



# THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME 154

155

*July-September 1948*

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 154

NUMBER 1

## METABOLISM OF THYROXINE IN THE GOAT<sup>1</sup>

R. A. MONROE AND C. W. TURNER

*From the Department of Dairy Husbandry, University of Missouri*

COLUMBIA, MISSOURI

**D**URING the past few years great progress has been made concerning the metabolism of the steroid hormones, especially the estrogens, androgens and progesterone. It has been shown that these hormones are metabolized by a variety of mechanisms in preparation for their elimination. The changes effected in the hormones—including the conversion to less active or inactive forms, and conjugation with glucuronidates or sulfates—are generally believed to occur in the liver.

The problem of the metabolism of thyroxine, on the other hand, has not received the study that its importance merits, due primarily to the fact that suitable methods for such investigations had not been developed until recently. However, many valuable inferences may be drawn from the older literature on iodine metabolism.

It may be said that, in general, the liver is primarily concerned with handling the more complex (organic) iodine compounds in contrast to the kidney, which is concerned mostly with the simpler (inorganic) iodine compounds. Consequently, the liver apparently plays an important rôle in the metabolism of the thyroid hormone as well as of the sex hormones. As early as 1919, Kendall (1) injected massive doses of thyroxine intravenously in the dog and found that 43 per cent of the iodine so administered was excreted in the bile in 50 hours. It is not known whether this iodine was in the form of thyroxine or of some decomposition product. A year later, however, Blum and Grützner (2) showed that the liver has the power to inactivate the thyroid hormone.

Some of the earlier workers felt that the hormone was totally inactivated by the liver (3-5). However, their observations were based on rather insensitive biological tests. Zawadowsky and

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Received for publication June 3, 1948.

<sup>1</sup> Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 1115.

Asimoff (6), on the other hand, suggested that the thyroid hormone is excreted by the liver unchanged. Their assay method (axolotl metamorphosis) was somewhat more sensitive, but not enough so as to make their observations conclusive.

Probably the truth of the matter lies somewhere between these two extremes. The work of Asimoff and Estrin (7) and Krayner (8) support the idea that thyroxine is only partially destroyed by the liver. Also, several investigators have shown, by chemical fractionation, that about one half to two thirds of administered thyroid hormone may be recovered in the bile (9-12). Kellaway *et al.* (13) are of the opinion that the thyroxine inactivating mechanism of the liver depends on the dosage of the hormone administered. Only when there is an excess of hormone, they say, is the destructive mechanism brought into play; and they stress the opinion that the mechanism is one of destruction rather than one of simple excretion. Presumably, on this basis, the amount of destruction would also depend on the dosage of the hormone administered.

Apparently, therefore, when thyroid hormone is administered to an animal, it may be partially broken down in the liver and partially excreted intact into the bile. As far as is known, no other organ or organ system contributes greatly to either the metabolism or excretion of thyroxine.

The recent work of Gross and Leblond (14), who used thyroxine labelled with radioactive iodine, bears out these postulations. In addition, these investigators found that much of the injected dose of thyroxine can be found *per se* in the feces, an observation which had been suggested previously (8, 15) but had not been emphasized in the literature.

Some results obtained at this laboratory also indicate that thyroxine is excreted intact in the feces by the goat. Since so little has been said on this subject in the literature, it seemed worthwhile to report our results at this time.

It was thought that the presence of thyroxine (or of some thyroidally active compound, at least) in the urine and/or feces could be detected by incorporating these excreta in chick rations simultaneously with thiouracil. The thiouracil, of course, would cause thyroid hypertrophy in the chick, due to the prevention of thyroxine synthesis and the consequent increase in thyrotrophic hormone secretion. It follows, therefore, that a material reduction of such hypertrophy would indicate the presence of a thyroidally active substance. An experiment was set up on this general hypothesis.

#### PROCEDURE

The urine and feces of 2 female, lactating goats were collected for five days. The urine was added to 10 kilos of poultry ration and the whole mixture dried in an oven at 45°C. The feces were dried at the same temperature, then ground and mixed in the feed at a level of 20 per cent by weight.

The goats were then injected subcutaneously with 10 mg. of D,L-thyroxine daily for a period of 10 days. This thyroxine was dissolved in N/10 sodium hydroxide and, therefore, was administered as the disodium salt.

The urine and feces were collected during the first five days of injections and were dried and mixed in the feed as described above. The same procedure was followed during the second five days of injections. Thiouracil<sup>2</sup> was added to each of these feeds at a level of 0.1 per cent by weight.

Eight groups of day-old White Rock chicks were used in this assay, each group consisting of about 20 chicks. One group received normal feed and served as a control. A second group received normal feed containing 0.1 per cent thiouracil. The remaining groups received the feeds containing the various samples of urine and feces described above (table 1). The assay was of three weeks' duration, starting on June 23 and ending on July 14. The chicks were kept in a basement room illuminated by incandescent bulbs and diffuse sunlight. The average daily temperature varied from 80-85°F.

<sup>2</sup> We are indebted to Lederle Laboratories, Pearl River, N. Y., for the thiouracil used in this experiment.

At the end of the experimental period the chicks were killed by ether asphyxiation, their body weights ascertained and the thyroid glands removed and weighed immediately. The sex of each chick also was determined at this time.

## RESULTS

The results of this experiment seem relatively clear cut. The addition of thiouracil to the normal chick ration, of course, caused marked thyroid enlargement (table

TABLE I. THYROIDAL ACTIVITY IN THE FECES OF GOATS INJECTED WITH THYROXINE

GROUP	FEED	MALES				FEMALES			
		No.	Body wt.	Thyroid wt.	Thyroid wt/100 gm. body wt.	No.	Body wt.	Thyroid wt.	Thyroid wt/100 gm. body wt.
			gm.	mg.	mg.		gm.	mg.	mg.
I	Control	9	148.8	4.9	$3.4 \pm 0.9$	12	159.3	7.9	$5.0 \pm 1.4$
II	Control + 0.1% thiouracil	11	138.6	42.5	$30.6 \pm 10.8$	7	132.1	48.7	$36.9 \pm 17.1$
III	Control + thiouracil + 14.8 l. normal goat urine/10 kg. feed	9	121.4	25.0	$28.9 \pm 12.1$	9	123.8	46.1	$37.6 \pm 10.9$
IV	Control + thiouracil + 8.4 l. goat urine <sup>1</sup> /10 kg. feed	8	138.0	27.5	$19.5 \pm 8.1$	12	143.2	52.0	$35.8 \pm 16.3$
V	Control + thiouracil + 10.3 l. goat urine <sup>2</sup> /10 kg. feed	11	161.7	48.5	$31.5 \pm 11.9$	8	146.4	57.0	$39.3 \pm 18.3$
VI	20% normal goat feces + thiouracil	12	146.8	32.2	$21.9 \pm 13.2$	7	143.7	49.9	$35.0 \pm 17.9$
VII	20% goat feces <sup>1</sup> + thiouracil	7	153.7	37.4	$23.1 \pm 10.4$	10	146.6	55.0	$36.4 \pm 20.9$
VIII	20% goat feces <sup>2</sup> + thiouracil	10	145.9	8.2	$5.8 \pm 4.9$	8	165.8	7.4	$4.1 \pm 2.1$

<sup>1</sup> Collected during first 5 days of injections.

<sup>2</sup> Collected during second 5 days of injections.

1). This hypertrophy was completely counteracted by the inclusion of goat feces collected during the second five days of the injection period. By feeding this sample of feces to thiouracil-treated chicks, the thyroid glands of the chicks were maintained at normal weight.

On the other hand, the feeding of normal goat feces and feces collected during the first five days of injections to thiouracil-treated chicks seemed to cause only a slight reduction in thyroid size. Moreover, this decrease is only apparent. Statistical analysis showed that the thyroids of chicks thus treated were not significantly smaller than those of chicks receiving thiouracil alone. The small decrease observed might well be accounted for by the fact that the nutritive value of the feed was probably lowered by the addition of feces. Also, the chicks ate slightly less of the feed containing feces than did the chicks given normal feed.

Likewise, the inclusion of urine, from either normal or injected goats, did not cause a reduction of thyroid hypertrophy except in one group. *Group IV*, male chicks, receiving thiouracil and urine (collected during the first five days of injections)

in their feed, had thyroid glands which, under statistical analysis, proved to be significantly smaller than the thyroids of male chicks receiving thiouracil alone. The female chicks receiving the same treatment were not likewise affected. Whether these results can be repeated remains to be seen.

#### DISCUSSION

Normally, the amount of total iodine excreted in goat feces is small—3  $\mu\text{g}$ . in 24 hours (16). Assuming all of this iodine to be in the form of thyroxine, the feeding of normal goat feces at a level of 20 per cent by weight would be the equivalent of feeding  $1.5\text{--}2.0 \times 10^{-6}$  per cent thyroxine. Since it takes about fifty times that amount of crystalline D,L-thyroxine in the feed to maintain the thyroid glands of thiouracil-treated chicks at a normal weight (17), it is not surprising that we find no thyroïdal activity in normal goat feces.

On the other hand, after thyroxine has been administered, even subcutaneously, there is an appreciable amount of the hormone passed into the feces. As shown above, enough hormone is present in the feces, collected during the second five days of injections, to maintain the thyroids of thiouracil-treated chicks at normal weight when the feces comprise 20 per cent of the feed. The same result can be effected by feeding the disodium salt of D,L-thyroxine<sup>3</sup> at a level of  $3.5 \times 10^{-5}$  per cent by weight (17). Therefore, it can be calculated that these feces contained approximately the equivalent of 0.35  $\mu\text{g}$ . of disodium D,L-thyroxine per gram of dried feces. On this basis, the daily excretion would be roughly 0.075 to 0.1 mg. of thyroxine per goat. This amount accounts for only one per cent or less of the daily injected dose. Moreover, subsequent experiments, although not conclusive, seem to indicate that the hormone is excreted at this rate for only a short time after the injections are stopped and that hormone excretion probably ceases altogether after a few days.

Obviously, much of the injected thyroxine remains to be accounted for. Presumably it is broken down in the body, probably in the liver for the most part. The mechanisms which the liver employs to accomplish this inactivation of thyroxine can only be speculated upon at this time.

Perhaps, since thyroxine is an amino acid, its inactivation follows the usual path of amino acid metabolism; i.e., deamination (probably oxidative), which might or might not be followed by a secondary reduction. The end product would then be an  $\alpha$ -keto acid or, if the secondary reduction did take place, an  $\alpha$ -hydroxy acid. Some credence is lent to this possibility by the observation of Foster and Gutman (19) that after the administration of massive doses of diiodotyrosine, a compound identified as 3,5-diiodo-4-hydroxyphenyl, lactic acid was found in the urine.

Other possible methods of thyroxine inactivation include: the release of iodine from the thyroxine molecule (12, 14) to form the inactive thyronine; the conversion of the physiologically active levorotatory isomer to the inactive dextro-isomer (20); and the breaking of one (or maybe even both) of the thyroxine rings (21).

At any rate, the amount of thyroïdally active material found in the feces in these experiments is much smaller than the amounts reported by other workers (8, 14). Krayner (8), of course, based his findings on the increment in fecal iodine after the injection of thyroxine; so we have no way of knowing, from his work, how much biologically active material was actually excreted. Gross and Leblond (14),

<sup>3</sup> Prepared by the method of Harington (18).

on the other hand, used thyroxine labelled with  $I^{131}$  and found that 80 per cent of the injected dose of radioactive thyroxine appeared in the feces in 24 hours. About one half of this amount was butanol soluble; i.e., was present as thyroxine. This recovered thyroxine, however, was found to be somewhat less active biologically than thyroxine prepared by the investigators or commercial preparations. They attributed this fact to either the presence of an inactive iodine compound dissolved in the butanol or to a toxic effect of the feces extract, which might cause a diminished metabolic response to thyroxine. It would be interesting to determine how much of the thyroxine is excreted in the d-form (inactive).

Concerning the mechanism of fecal excretion of thyroxine, there seem to be two major possibilities: *a*) the liver probably excretes some unchanged thyroxine into the bile, and hence into the digestive tract (8-12, 14); and *b*) the intestine may actively excrete some thyroxine (15). The former route is doubtless the more important (14).

It must be remembered, however, that the gastrointestinal tract also absorbs thyroxine. Probably the actual, overall picture is a complicated combination of excretion, reabsorption, and metabolism, the extent of each of these processes depending on various unknown factors.

#### SUMMARY

A study has been conducted on goats to determine whether thyroxine injected subcutaneously is excreted in the feces and/or urine in a biologically active form. It was found that unmistakable activity appeared in the feces of goats during the fifth through the tenth day of a 10-day injection period. Estimations of active material in the feces are presented in terms of the amount of disodium thyroxine required to evoke an equivalent response. No activity could be detected in normal goat feces or urine, in feces collected during the first five days of injections, or in urine collected at any time during the injection period.

Various possible mechanisms of thyroxine inactivation are discussed.

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# INTERRELATION BETWEEN THE LENGTH OF SYSTOLE, STROKE VOLUME AND LEFT VENTRICULAR WORK IN THE DOG<sup>1</sup>

JOHN W. REMINGTON, W. F. HAMILTON AND RAYMOND P. AHLQUIST

*From the Departments of Physiology and Pharmacology, University of Georgia School of Medicine .*

AUGUSTA, GEORGIA

IT IS well established that for resting human subjects, the Q-T interval of the electrocardiogram varies directly with the length of the cycle (1-4). Hence there seems to be a basic relation between the duration of the excitation process, and the frequency of pacemaker discharge. During periods of rapid change in cycle length, however, the relationship is less exact (4). When the duration of the actual ejection period is plotted against cycle length, for normal dog hearts in a wide variety of conditions, the correlation between the lengths of systole and cycle is very rough indeed (fig. 1). Factors other than cycle length must play a rôle in determining the length of the ejection period. Thus Wiggers, and Wiggers and Katz (5-7), have shown that an increase in filling pressure (right auricular pressure at the end of diastole) tends to increase the relative duration of systole and that an increased arterial pressure and sympathetic stimulation tend to decrease it. These observations were made on the exposed heart whose systolic duration was very much longer than that of the heart beating in the closed thorax. It was thought advisable, therefore, to inquire further into the factors affecting length of systole in the intact dog.

In a previous publication (8), we have shown that what can be learned from the heart lung preparation applies also to the intact animal, namely that an increase in arterial pressure serves to decrease the stroke volume and often the external cardiac work. Conversely, a fall in arterial pressure produces an increase in stroke volume. It was also confirmed for the intact animal that, within the limits set by the pericardium, a rise in venous pressure increases both stroke volume and external work. In the absence of sympathomimetic myocardial stimulation, which shortens the duration of systole, the data of Wiggers (5-7) would suggest, therefore, a possible parallelism between the length of systole, the stroke volume and external ventricular work.

As a first step in the delineation of the factors causing a change in length of systole in the intact animal, statistical analyses were made of values derived from some 800 dog aortic pressure pulse contours, taken from a number of animals, and chosen to represent a rather wide variety of experimental conditions. The lengths of systole were measured as the time from abrupt pressure rise to the incisural notch. These reference points were taken for convenience only. The length of systole so

Received for publication July 1, 1948.

<sup>1</sup> This research was supported by a grant from the Life Insurance Medical Research Fund, and from Ciba Pharmaceutical Products, Inc.

recorded is, of course, not an index to the duration of muscle contraction, for the isometric contraction period is not included. Neither does it coincide with the ejection period, for outflow from the ventricle may cease before the incisura. While this time difference is not necessarily constant, it is small so that, roughly, the length of systole as measured will reflect changes in the duration of ejection.

The length of systole values ( $T_s$ ) were plotted against the respective cycle lengths (fig. 1 A). The points are widely scattered about a curved line that was plotted from average  $T_s$  values within cycle length groups. This curve is not fitted, over its whole length, by exponential equations similar to those used to relate the length of electrical systole to cycle length (1, 4). The correlation coefficient for the data of figure 1 A is only 0.62. The curved relation renders most questionable the significance of this figure. The correlation coefficient with the square root of cycle length, or with the reciprocal of cycle length, i.e., pulse rate, is higher ( $r = 0.75; 0.77$ ).

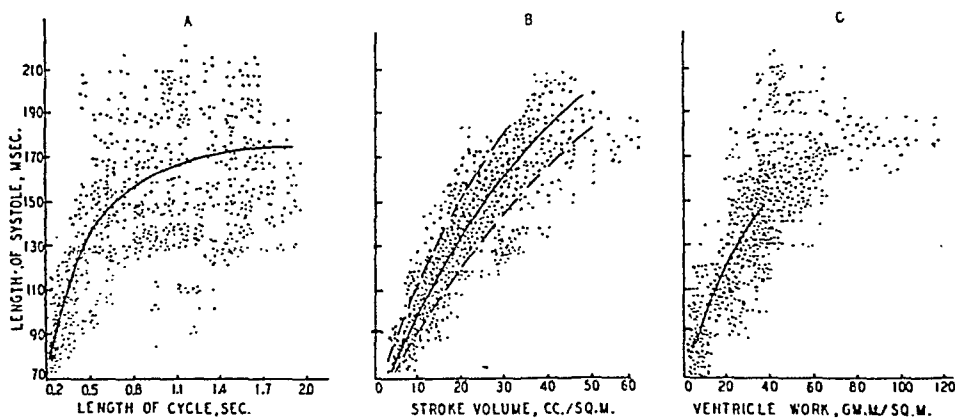


Fig. 1A. RELATION BETWEEN LENGTHS OF SYSTOLE AND CYCLE LENGTHS of dog pulses. 1B. Relation between lengths of systole and stroke volumes for the same series of pressure pulses. Dotted lines represent 25 per cent deviation from the average curve. 1C. Relation between lengths of systole and work of the left ventricle.

The scatter of the points is greater than that recorded for electrical systole. This is partly because heart rate changes are greater and physiological conditions more variable in the present series. Moreover, electrical criteria measure only the duration of the process of excitation of the myocardium. The length of mechanical systole, while it has a similar trend to that of Q-T, is modified by conditions imposed upon the heart as it attempts to eject blood against a variable level of aortic pressure (5-7).

In other words, under certain conditions there is an even greater shortening of mechanical systole than a cardio-acceleration will account for. Also, when the heart is slowed, the length of systole may remain the same or lengthen with the cycle length. As a measure of this dispersion, we have taken the average curve of figure 1 A as the comparison standard (table 1) and divided the actual length of a particular systole by the value expected from the length of the cycle, expressing the quotient as a percentage ( $T_s/T_{s_e} \times 100$ ). This figure will express the effect of mechanical variables upon  $T_s$ , discounting in advance the effect of heart rate changes.

It should be emphasized that the figure for  $T_s/T_{s_e}$  is of significance only as it changes. It represents the relation of an actual  $T_s$  to an average at that particular



heart rate. This average is derived from pulses from different physiological states and is weighted heavily by pulses from partly deteriorated animals. In 43 newly anesthetized animals,  $T_s/T_{s_0}$  averaged 113 with a standard deviation of 10. As the animal deteriorates during an experiment, this value falls progressively to 100, or below. Likewise, an animal in shock shows low values (11).

The observation of Wiggers that increased aortic pressure decreased the duration of systole justifies inquiry as to whether blood pressure is itself one of the factors which produced trends away from the average in figure 1 A. The correlation between  $T_s$  and the height of systolic and diastolic pressure levels was found to be 0.28 and 0.36 respectively. It would seem, therefore, that the effect of pressure level on systole length was not a primary relation. On the other hand, the correlation between  $T_s$  and stroke index of these dogs, either as calculated from the pulse contour (9) or as obtained by the dye injection technique (10), is quite high ( $r = 0.82$ ). The relation of the individual measurements is shown in figure 1 B. The scatter is not large, and the stroke index can be predicted from  $T_s$  with an average error of 25 per cent.

TABLE 1. AVERAGE RELATION BETWEEN LENGTH OF CYCLE AND LENGTH OF SYSTOLE

LENGTH OF CYCLE	LENGTH OF SYSTOLE ( $T_{s_0}$ )	LENGTH OF CYCLE	LENGTH OF SYSTOLE ( $T_{s_0}$ )	LENGTH OF CYCLE	LENGTH OF SYSTOLE ( $T_{s_0}$ )	LENGTH OF CYCLE	LENGTH OF SYSTOLE ( $T_{s_0}$ )
msec.	msec.	msec.	msec.	msec.	msec.	msec.	msec.
250	84	500	134	750	155	1200	168
300	99	550	141	800	157	1400	172
350	110	600	145	900	161	1600	174
400	119	650	149	1000	164	1800	175
450	127	700	152				

A similar correlation obtains between  $T_s$  and pulse pressure (0.79) and between  $T_s$  and left ventricular external work (0.86) (fig. 1 C). The high correlation with pulse pressure may be regarded as a corollary of the high correlation with stroke volume. The high correlation with external work may be either direct or indirect through the relation with stroke volume. A case could be presented for either view point. For example, if the above series be subdivided into two groups, those with high and those with low diastolic pressure values, the correlation between  $T_s$  and stroke index is essentially the same for both groups. That, between  $T_s$  and work, however, is definitely better ( $r = 0.92$ ) for the high-pressure pulses and definitely inferior ( $r = 0.63$ ) for the low-pressure pulses.

For an analysis of this difference with pressure levels, we have followed the immediate changes in stroke volume, work and  $T_s/T_{s_0}$  when cardiodynamics were abruptly altered by various experimental procedures.

From table 2 A-C it is seen that when the abdominal aorta, just below the diaphragm, was occluded the stroke volume, work and  $T_s$  were at first reduced. As the occlusion persisted all three might return toward the initial level. With marked cardiac slowing, the work and  $T_s/T_{s_0}$  might exceed the initial value (2 C). In other cases, all quantities might remain low throughout the occlusion (2 B).

From the average stroke volume and  $T_s$  relation (fig. 1 B), one can predict the stroke volume which should correspond to each  $T_s$  value. From such values (table 2), it is apparent that, when the heart is faced with a high aortic resistance, the stroke volume is curtailed to a much greater degree than is  $T_s$ . Not only did  $T_s/T_{s_e}$  decline less than stroke volume or work but also the change was of briefer duration, so that  $T_s/T_{s_e}$  might return to the initial value while the other measures were still reduced (table 2 A).

A number of constrictor drugs which, in our hands, have had a minimal effect upon the heart gave similar changes in  $T_s$ , work and output. As the pressure first rose after the injection of angiotonin<sup>2</sup> (table 2 D) or Privine (table 2 E), the stroke volume and work were reduced and  $T_s/T_{s_e}$  was shortened slightly. Later the work returned toward normal and  $T_s/T_{s_e}$  lengthened, even though there had been no increase in stroke volume. After Neosynephrine (table 2 F) neither work nor  $T_s/T_{s_e}$  decreased, even though the pressure was increased and the stroke volume decreased.

In all these experiments in which the heart was suddenly faced with an increased aortic resistance to ejection,  $T_s$ , stroke volume and work were immediately curtailed. After this initial transient change,  $T_s$  returned to the expected value and then varied with cycle length but not with stroke volume or work.

Another group of sympathomimetic drugs which bring about their pressor effects by increasing the cardiac output allow a definite increase in  $T_s/T_{s_e}$ . Thus Prisol (table 2 G) and ephedrine (table 2 H) produced a decided increase in work per beat and in  $T_s/T_{s_e}$ . In some cases there was a moderate increase in stroke volume as well. In all these cases, the changes in  $T_s/T_{s_e}$  paralleled those of work more than those of stroke volume.

When the aortic pressure was suddenly reduced, the work might or might not be increased as the stroke volume was increased. For example, when an aortic occlusion was released (table 3 A), or when a shunt between the abdominal aorta and vena cava was opened (table 3 B), both stroke volume and  $T_s/T_{s_e}$  were abruptly increased. Work changes were rather variable, depending upon the pressure levels. In the first case, for example, the work was also momentarily increased; in the second, it was not. The limitation of cardiac ejection with a low aortic pressure depends primarily on the adequacy of venous filling rather than aortic resistance and the ventricle can easily eject its whole possible stroke volume. The presence of a reactive hyperemia in the peripheral beds, for example, allows less of an increase in  $T_s$  than does the opening of an A-V shunt or the injection of acetylcholine. The fact that the external work may not numerically reflect the increased ejection, and hence be no criterion to  $T_s/T_{s_e}$ , need not be surprising.

With extremely long cycle lengths, as during the stimulation of the peripheral end of the vagus (3 C),  $T_s$  might remain relatively independent of cycle length, and  $T_s/T_{s_e}$  thereby be reduced.  $T_s$  itself was lengthened as the stroke volume increased and in about the ratio expected from figure 1 B. Both values reached the maximum available for this animal under the existing conditions and, beyond a certain level, a

<sup>2</sup> We are indebted to Dr. F. F. Yonkman for the prisol and Privine, to Dr. A. M. Lands for the Neosynephrine and the N-isopropyl arterenol, and to Dr. O. M. Helmer for the angiotonin used in this study.

TABLE 2

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	Ts/T <sub>sc</sub>	EXPECTED <sup>1</sup> STROKE INDEX
<i>A—Aortic occlusion, after atropinization</i>							
<i>sec.</i>	<i>mm. Hg</i>	<i>cc.</i>	<i>gm. M.</i>	<i>msec.</i>	<i>msec.</i>	<i>%</i>	<i>cc.</i>
0	126/ 93	18	30	120	400	101	15
3	186/154	5	14	110	400	93	13
5	196/162	6	15	115	400	96	14
10	195/160	6	15	117	400	97	14
<i>B—Aortic occlusion</i>							
0	102/ 77	21	26	140	390	120	22
3	200/156	4	10	125	390	107	17
5	210/163	8	20	140	420	115	22
10	210/158	9	20	150	510	112	24
<i>C—Aortic occlusion</i>							
0	140/118	18	33	140	600	97	22
2	170/140	15	27	130	580	90	18
5	264/160	16	50	180	800	115	37
10	240/160	12	38	160	800	103	28
<i>D—Angiotonin, 10 units</i>							
0	110/ 88	14	19	125	350	113	25
5	140/126	11	17	115	350	104	24
10	148/134	10	20	120	340	111	32
15	152/134	11	20	127	340	117	27
<i>E—Privine, .02 mg/kg. B.W.</i>							
0	162/132	13	22	90	270	100	8
10	204/166	7	15	86	280	90	7
20	190/150	8	16	95	270	105	9
<i>F—Neosynephrine, .02 mg/kg. B.W.</i>							
0	140/116	13	17	130	440	104	18
10	176/148	10	17	140	480	109	21
<i>G—Priscol, 10 mg/kg. B.W.</i>							
0	164/135	13	28	150	580	104	24
10	176/140	15	35	160	600	110	28
30	184/145	17	38	165	620	113	30
50	193/147	18	42	170	620	116	32
<i>H—Ephedrine, 5 mg/kg. B.W.</i>							
0	130/104	16	25	90	280	95	9
5	140/116	15	27	90	270	100	9
10	156/120	16	32	100	240	120	10
15	158/122	17	32	110	280	114	13

<sup>1</sup> Stroke index expected from length of systole (fig. 1 B).

further increase in diastolic time did not produce an appreciable increase in either factor.

When low arterial pressure was produced by acetylcholine injection (3 D, E),  $T_s$  tended to increase as the stroke volume increased, while the work changes were

TABLE 3

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	$T_s/T_s:$	EXPECTED STROKE INDEX
<i>A—Release of aortic occlusion</i>							
<i>sec.</i>	<i>mm. Hg</i>	<i>cc.</i>	<i>gm. M.</i>	<i>msec.</i>	<i>msec.</i>	<i>%</i>	<i>cc.</i>
0	226/176	8	20	115	430	93	15
5	122/106	33	43	130	420	107	18
10	110/92	27	27	124	410	103	17
15	100/84	25	25	120	400	101	16
<i>B—Abdominal aorta—vena cava shunt</i>							
0	128/92	24	22	170	550	120	33
1	62/40	40	16	190	550	135	42
2	82/28	42	14	198	560	141	46
3	86/36	48	18	200	570	141	47
<i>C—Stimulation of peripheral end of vagus nerve</i>							
0	70/48	11	9	82	250	98	6
5	75/20	29	30	138	1250	82	21
10	92/30	36	33	140	1750	80	21
15	82/38	29	25	130	1250	77	18
<i>D—Acetylcholine, .004 mg/kg. B.W.</i>							
0	120/95	21	30	125	340	116	17
2	100/70	23	26	120	320	117	16
5	90/58	27	29	130	290	136	18
<i>E—Acetylcholine, .02 mg/kg. B.W.</i>							
0	105/80	20	25	120	360	107	16
2	64/20	30	17	160	900	100	28
5	76/28	33	23	160	690	106	28
8	82/58	26	25	140	290	142	21

variable. Just as with vagus stimulation, with very long cycle lengths,  $T_s/T_{s_0}$  might be reduced.

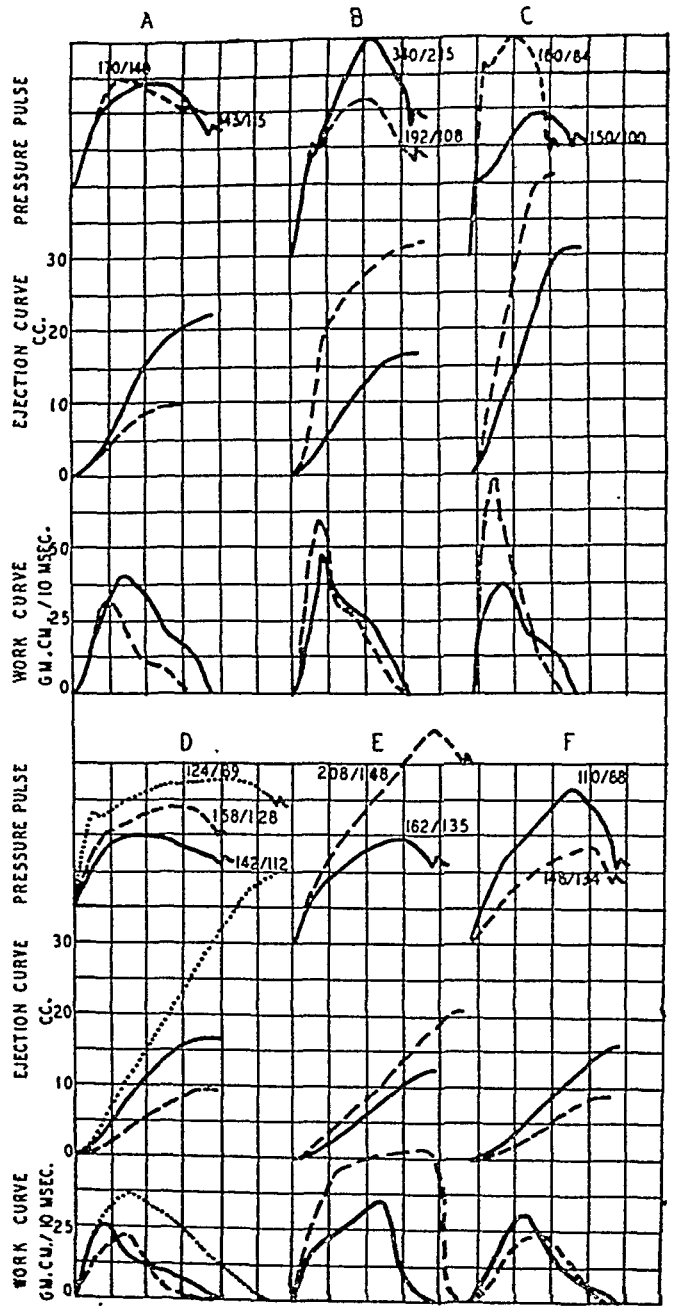
As shown by Wiggers (6), sympathetic stimulation of the heart gives rise to an abridgement of systole. The stimulation of the sympathetic nerves to the heart (table 4 A) gave a cardio-acceleration and increased stroke volume and ventricular work, but a decreased  $T_s/T_{s_0}$ . This effect is directly opposed to the  $T_s/T_{s_0}$  response to be expected when the work per beat is increased. Similarly, injected epinephrine gave a reduced  $T_s/T_{s_0}$  (table 4 B). As we have shown (8), the usual response to epinephrine in the dog is to reduce the stroke volume and external work, since the vasoconstriction outweighs the cardiac stimulation.

TABLE 4

TIME	BLOOD PRESSURE	STROKE INDEX	WORK	LENGTH OF SYSTOLE	LENGTH OF CYCLE	Ts/Tsc	EXPECTED STROKE INDEX
<i>A—Stimulation of cardiac nerves</i>							
<i>sec.</i>	<i>mm. Hg</i>	<i>cc.</i>	<i>gm. M.</i>	<i>msec.</i>	<i>msec.</i>	<i>%</i>	<i>cc.</i>
0	170/134	15	31	148	460	116	24
5	190/146	16	38	136	430	109	20
10	200/154	18	43	132	425	107	19
20	198/148	15	35	135	435	108	20
<i>B—Epinephrine, .005 mg/kg. B.W.</i>							
0	143/115	20	37	120	400	101	16
5	156/130	11	20	95	320	92	9
10	168/137	10	22	108	600	75	12
15	160/118	17	35	150	800	96	24
25	132/107	21	38	130	500	97	18
35	130/101	24	40	130	400	109	18
<i>C—Epinephrine, .002 mg/kg. B.W.</i>							
0	165/108	27	50	170	900	106	33
10	168/108	27	50	120	760	78	15
20	176/108	31	58	130	660	88	18
<i>D—Epinephrine, .015 mg/kg. B.W.</i>							
0	133/105	22	36	140	370	123	21
5	235/180	13	37	120	350	109	15
10	300/210	14	51	140	350	127	21
<i>E—Epinephrine after prisol, .005 mg/kg. B.W.</i>							
0	180/138	17	39	148	520	109	24
10	209/145	17	44	110	490	83	13
20	170/115	25	46	100	380	87	11
30	148/104	27	49	90	400	84	11
<i>F—N-isopropyl arterenol, .0025 mg/kg. B.W.</i>							
0	150/100	26	44	140	420	116	21
5	156/ 90	33	55	110	350	100	13
10	143/ 70	47	65	100	280	107	11
15	130/ 60	29	37	90	260	103	8
<i>G—N-isopropyl arterenol, .0002 mg/kg. B.W.</i>							
0	165/122	16	33	140	520	103	21
5	167/125	16	33	100	360	89	11
10	158/118	15	30	100	350	91	11
15	166/125	16	33	110	420	91	13
20	165/125	16	33	130	470	99	15

Cases where the stroke volume reduction is minimal, or absent, (4 C) still show an abridgement of  $T_s/T_{s_0}$ . That this shortening is due to a direct action of the drug, and not to the mechanical effect of the rise in pressure, was shown by several experiments. In some cases, the effect upon the blood pressure (and hence upon the stroke

Fig. 2. RELATIONS between pressure pulses, derived ejection curves and work curves for dog pulses in various physiological conditions. A—Before (*solid*) and after (*broken*), the injection of epinephrine. B—Before (*solid*) and after (*broken*) the opening of an aorta-vena cava shunt, at the height of the pressure rise following the injection of epinephrine. C—Before (*solid*) and after (*broken*) the injection of isopropyl arterenol. D—Before (*solid*) the occlusion of the abdominal aorta, during (*broken*) that occlusion, and immediately after (*dotted*) the release of the occlusion. E—Before (*solid*) and after (*broken*) the injection of Priscol. F—Before (*solid*) and after (*broken*) the injection of angiotonin.



volume and work output) outlasted that upon systolic length, which might return to its pre-injection value even though the stroke volume was still curtailed by the high pressure (4 D). When, at the height of the pressure rise, the peripheral resistance was suddenly lowered by the opening of a previously established shunt between the aorta and vana cava, the length of systole did not increase, although the stroke vol-

ume and external work were increased to a great degree. Small doses of epinephrine might cause a reduction in  $T_s/T_{s_0}$  even when there was no effect upon the blood pressure or blood flow (table 4 C). When epinephrine was given after Priscol, a drug which blocks the vasoconstrictor action, the length of systole was still reduced (table 4 E) even during the period in which the stroke volume was increased and the blood pressure was falling.

N-isopropyl arterenol, which possesses the cardiac actions of epinephrine, but is a peripheral vasodilator (8, 12) will, in minimal doses, give a reduction in the length of systole, a cardio-acceleration, and no change in blood pressure or stroke volume (table 4 G). In larger doses, it curtailed  $T_s/T_{s_0}$  in spite of an increase in stroke volume and ventricle work (table 4 F).

Hence the reduction in  $T_s$  seen after cardiac nerve stimulation, or use of epinephrine-like compounds, is due to a direct action on the time course of contraction and is not to be attributed to the influence of mechanical factors upon the performance of the ventricle. This is shown graphically by the cardiac ejection curves which can be constructed from the aortic pressure pulses (13). For example, in figure 2 A are given representative pulse contours taken before and during the first part of the response to epinephrine, with their derived ejection curves, and also the curves depicting the work done during the course of systole (14). The changes are quite similar to those obtained by Wiggers with a cardiometer when epinephrine was given (6). After the drug, the time course of ejection was altered, so that the maximum output came earlier in systole, and both ejection and external work fell off rapidly during the last two thirds of the ejection period. The myocardial stimulation is hence revealed in a more forceful initial contraction, but a contraction which can be but poorly sustained.

That the changes pictured cannot be attributed simply to the reduced stroke volume and external work is evident from figure 2 B and C. In figure 2 B a previously established A-V shunt was opened at the height of the pressure rise after the injection of epinephrine. As the pressure was suddenly lowered, stroke volume and work were increased, but the contour of the work curve showed but minor variation. In figure 2 C, N-isopropyl arterenol was used to produce the cardiac stimulation. Both stroke volume and cardiac work were enhanced. Despite this, the maximal ejection rate and work production were reached earlier in systole after its use, and, like the response to epinephrine, both work and ejection were but poorly sustained toward the end of systole.

On the isolated heart, the actions of these two drugs are identical. In the intact dog, N-isopropyl arterenol, being a peripheral dilator instead of constrictor, always increases the stroke volume. Epinephrine, because of its intense peripheral constriction, usually leads to a decreased stroke volume in the dog.

Work contour changes which follow a rise in aortic pressure do not show the above changes. After the application of an aortic occlusion (fig. 2 D), or the injection of angiotonin (fig. 2 F), there was a broadening of the work curve, with the peak falling later in systole. Changes which followed the release of an aortic occlusion (fig. 2 D), or the injection of Priscol (fig. 2 E), were quite similar. In these cases, the cardiac work was increased rather than decreased,  $T_s/T_{s_0}$  was lengthened and

the ventricle actually worked strongly for almost the whole of the ejection period. The pressure pulse contours reflect this change, showing a pressure peak late in systole. After epinephrine, the pressure peak is reached in mid- or early systole.

#### SUMMARY

1. Mechanical factors upset a natural relation between length of systole and cycle length. Hence length of systole shows good correlation with stroke volume and with left ventricle work in the dog. 2. Systole is longer than might be expected from cycle length when the arterial pressure is low and venous return to the heart is adequate. This increase parallels the increase in stroke volume. Systole is also prolonged when the heart is stimulated by ephedrine or Priscol to do more work. 3. Systole is shorter than might be expected from cycle length, very transiently, when there is a sudden increase in aortic resistance to ejection. A more prolonged curtailment of systole follows cardiac nerve stimulation, or the injection of epinephrine or related compounds. This change reflects a basic change in the ejection curve.

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# CHOLESTEROL AND CAPILLARY PERMEABILITY<sup>1</sup>

ALENE F. SILVER<sup>2</sup>

*From the Department of Physiology, University of Illinois*

CHICAGO, ILLINOIS

MUCH past research has ascribed to cholesterol the function of regulating cellular permeability (1-4), but these claims were largely inferential, or based on evidence from work with single cells or isolated tissues. It was our purpose to see if cholesterol has any effect on capillary permeability, as tested in a total mammalian animal.

## PROCEDURE

Changes in capillary permeability were tested by the transfer of intravenously injected dye, T-1824, from the blood to the lymph, and also by the disappearance rate of the dye from the blood stream. Twenty-four dogs were used, comprising 10 untreated controls, 4 controls treated with water and 10 experimental animals treated with cholesterol.

All finally underwent the same procedure. The dog, fasted for 20 hours, was intravenously anesthetized with nembutal, 30 mg./kg. and the thoracic duct exposed and cannulated. The lymph was collected continuously in test tubes and the mean time of collection was noted for each test tube. After control specimens of blood and lymph were obtained, a measured amount of dye was injected into the exposed femoral vein. Lymph specimens were timed at 5, 15, 30, 45, and 60 minutes following dye injection. Blood samples from the contralateral femoral vein were taken at 15, 40, and 60 minutes after dye injection.

## METHODS

*Treatment of blood and lymph specimens.* Blood samples were centrifuged immediately after withdrawal, the relative amounts of cells and clotted plasma measured for hematocrit determination, and serum expressed by pressure. Lymph volumes were noted and fluid lymph expressed from the clot. All serum and lymph specimens were then treated with alcoholic phosphotungstic acid (5) to remove proteins and lipids. The dye content of these samples was determined by a photometer. Total serum cholesterol was determined by the Leiboff modification of the Myers-Wardell method (6).

*Administration of cholesterol.* Cholesterol suspensions were made following the method of Cole, Clarke and Womack (7). In some cases sodium laurylsulphonate was used as a wetting agent in order to produce a finer suspension. This proved ir-

Received for publication May 24, 1948.

<sup>1</sup> A part of the cost of this investigation was borne by the Arthritis Research Project of the University of Illinois.

<sup>2</sup> This material was presented in the thesis for the partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

ritating to the tissues and was discontinued. The experimental dogs received 40 to 60 cc. of suspension of a strength approximately 2.5 gm. per cent, intraperitoneally, each day for one week preceding the operation. Sterile precautions were observed.

*Administration of water.* Four dogs received the same treatment as the experimental dogs except that they received a mock 'suspension' of water in place of the cholesterol suspension.

### RESULTS

*Cholesterol findings.* The control range of serum cholesterol in the untreated group was 43 to 196 mg. per cent, in the water-treated group 50 to 102 mg. per cent before the administration of water. For the experimental animals before treatment, this value was 45 to 148 mg. per cent. The entire group of 21 control determinations presented a range of 43 to 196 mg. per cent, with a mean of 95 mg. per cent, and

TABLE 1. EFFECT OF CHOLESTEROL ON VARIOUS FUNCTIONS RELATED TO CAPILLARY PERMEABILITY

FUNCTION TESTED	CONTROL GROUP			EXPERIMENTAL GROUP		
	No. of cases	Mean	s.d.	No. of cases	Mean	Dev. from control mean
Dye disappearance rate (%).....	14	6.6	4.5	10	6.2	-0.4
Blood vol. (cc/kg.).....	13	90	9.1	10	85	-5.0
Hematocrit (%).....	14	44	6.2	10	46	+2.0
Lymph flow (cc/kg.).....	14	.035	.023	9	.031	-.004
Transfer of dye to lymph:						
a. Time of maximum dye conc. in lymph (min.).....	14	43	14.8	10	33	-10
b. Maximum dye conc. in lymph/plasma dye conc. (15').....	14	.414	.180	10	.433	+.019
c. Total dye in lymph (60')/plasma dye conc. (15').....	14	.082	.072	10	.096	+.013

standard deviation of 35.6 mg. per cent. Glusker (8) found a standard deviation of 25 per cent for a series of normal dogs.

The 4 dogs treated with water evinced essentially no change in serum cholesterol. Before and after treatment the percentage change in cholesterol for each animal ranged from -2 to +9 per cent, the mean percentage change being +2 per cent, with a standard deviation of 4.6 per cent. Glusker gave 6 per cent as the standard deviation for 4 determinations on single dogs.

The 10 dogs treated with cholesterol evinced a rise in serum cholesterol in each case. The experimental range was 77 to 208 mg. per cent, with a mean of 156 mg. per cent, exhibiting a difference of +61 mg. per cent over the control mean of 95 mg. per cent, a difference almost twice as great as the standard deviation. The percentage change in cholesterol level for each case ranged from +8 to +276 per cent. The mean change was +76 per cent, to be compared with the mean change of +2 per cent for the water-treated animals. This difference was 16 times as great as the standard deviation, so that the rise in serum cholesterol after administration of cholesterol was unquestionably significant.

*Experimental findings in regard to capillary permeability.* In the case of all the functions investigated, the findings were negative. There was no statistically significant difference between the control group of untreated dogs plus water-treated dogs and the experimental group of cholesterol-treated dogs in regard to; a) disappearance rate of the dye from the blood stream, b) blood volume, c) hematocrit, d) lymph flow, e) transfer of dye to the lymph, either in regard to time of appearance, concentration achieved, or absolute quantities recovered in the lymph (table 1).

The correlation between individual cholesterol levels and dye disappearance rates was low ( $r = .21$ ). The correlation between individual cholesterol levels and dye transfer to the lymph was absent ( $r + .077$ ).

#### DISCUSSION

The method was found in the following paper (9) to be adequate for detecting marked changes in capillary permeability. In this case the experimentally induced rise in serum cholesterol, while not drastic, was of an order which might reasonably be expected to produce changes, if these were to be found. The consistently negative results found in this work, together with the failure of other investigators to find direct evidence of a dynamic effect of cholesterol on permeability, seems to lead inevitably to the negative conclusion.

#### SUMMARY

Intra-peritoneal injections of cholesterol suspension raised the plasma cholesterol level of dogs. An increased plasma cholesterol level had no effect on capillary permeability, as tested by the disappearance rate of dye from the blood stream and the transfer of dye from blood to lymph. In addition, no effect was found in the case of blood volume, hematocrit and lymph flow.

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# VITAMIN D AND THE DISAPPEARANCE OF T-1824 FROM THE BLOOD<sup>1</sup>

ALENE F. SILVER<sup>2</sup> AND C. I. REED

*From the Department of Physiology, University of Illinois*

CHICAGO, ILLINOIS

MANY substances are known to increase the permeability of capillary endothelium (1-5). Substances which can counteract this process, producing a condition of decreased permeability of capillaries to large water-soluble molecules, are less well known. Calcium and the adrenal cortical hormones (7-9) are of the latter category, and preliminary experiments in this department (10) indicated that vitamin D might also be included. Later work was confirmatory, both as to substance and method, and the final results are reported here.

## METHODS

Adult male dogs weighing 10 to 20 kg. were used. The disappearance rate of the dye T-1824 was used as the indicator of capillary permeability. Dye disappearance rate was determined by a technique similar to routine determinations of plasma volume and was calculated as the percentage difference in dye concentration between 15 and 60 minutes after dye injection.

In acute experiments, where lymph from fasting dogs was analyzed for dye content, the collected lymph was allowed to clot, compressed to express the dyed fluid, and the specimens then read on a photoelometer exactly as were the serum samples, since under these conditions the optical densities of serum and lymph were similar.

Vitamin D was administered orally in capsules of 50,000 International Units (IU). When histamine was employed, it was injected intravenously, using 1.5 mg/kg. of body weight.

### *Effect of Vitamin D on Capillary Permeability*

*Procedure and results.* Trained unanesthetized dogs were tested in several control determinations of dye disappearance rate, plasma volume and hematocrit. Vitamin D was then administered, and the determinations were continued for a varying period thereafter. The preliminary work had shown that lower or less concentrated doses of the vitamin had no uniform effect. Doses used had been a) 11,000-16,000 IU/kg/day for one to four days, and b) 2500-11,000 IU/kg/day for 40 days.

Positive results were obtained when a total dose of approximately 100,000 IU/kg. was administered over one to seven days. This dose was administered to 18 dogs, and repeated one week later in the case of 12 of the animals. The experimental

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Received for publication May 24, 1948.

<sup>1</sup> A part of the cost of this investigation was borne by the Arthritis Research Project of the University of Illinois.

<sup>2</sup> This material was presented in the thesis for the partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1947.

changes described below were detected in the determinations which were made within a period of from five to eight days after discontinuing the dosage. Both before and after this period, the experimental values approached the control level.

TABLE 1. EFFECT OF MASSIVE DOSE OF VITAMIN D

CASE	CHANGE IN BODY WT.	CHANGE IN DYE DISAPPEARANCE RATE	CHANGE IN PLASMA VOL.	CHANGE IN HEMATOCRIT	CHANGE IN BLOOD VOL.
	% <sup>1</sup>	% <sup>1</sup>	% <sup>1</sup>	% <sup>1</sup>	% <sup>1</sup>
4650a	+5	-125	+7	+8	+13
4848a	+3	-100	-6	+7	-4
4656a	-2	-180	-4	+7	+2
7	-4	0	-5	+10	+5
3	-7	-114	+60	+6	+71
4848b	-7	+50	-7	+19	+2
2a	-8	-87	+22	+15	+45
8	-8	-200	+4	+11	+15
4653a	-9	-67	-5	+17	+8
4654a	-9	-100	-21	+13	-11
4658b	-9	+33	-17	+16	0
9a	-9	0	—	+16	—
4659b	-10	-40	+18	+12	+28
4650b	-11	-75	-20	+28	-3
4655a	-11	-69	-23	+14	-10
4734a	-11	—	-14	+15	+3
4	-13	-170	+39	+16	+43
6	-13	-743	+23	+12	+35
4656b	-13	-80	-16	+25	+4
4659a	-13	-50	-8	+7	-4
4658a	-14	0	-3	+10	+8
2b	-16	-127	+25	+33	+71
9b	-17	+115	-28	+24	-16
4652a	-18	-44	-4	+21	+5
4654b	-18	-29	-21	+22	-4
4655b	-18	-62	-24	+27	+7
4653b	-19	-67	+1	+33	+33
5	-20	+218	-39	+11	-34
4734b	-23	-57	-42	+21	-13
4652b	-24	-28	-15	+64	+14
MEAN.....	-11.5	-66	-4	+18	+11

Single dose of 100,000 IU/kg., followed by latent period of one week (approx.).

<sup>1</sup> Based on deviation of experimental datum from last control datum. Omitting two extreme values.

It was found that vitamin D administration was followed by a decrease in capillary permeability, as measured by the dye test. Table 1 gives the percentage change in the various functions found after the latent period, the experimental datum in each case being compared to the last control datum before dosing. Out of a total of 29 tests of dye disappearance rate, 22, or 76 per cent showed a decrease in the rate at

which dye left the blood stream. The mean percentage change in disappearance rate, omitting two extreme values, was  $-66$  per cent.

There was a rise in the hematocrit value in each of the 30 cases tested (mean increase was  $+18\%$ ). This rise was not solely a passive reflection of plasma loss, as may be seen from the fact that the blood volume did not decrease, but rather the reverse (mean change in blood volume was  $+11\%$ ). Twenty cases showed a decrease in plasma volume, and 9 cases an increase (mean change was  $-4\%$ ). The

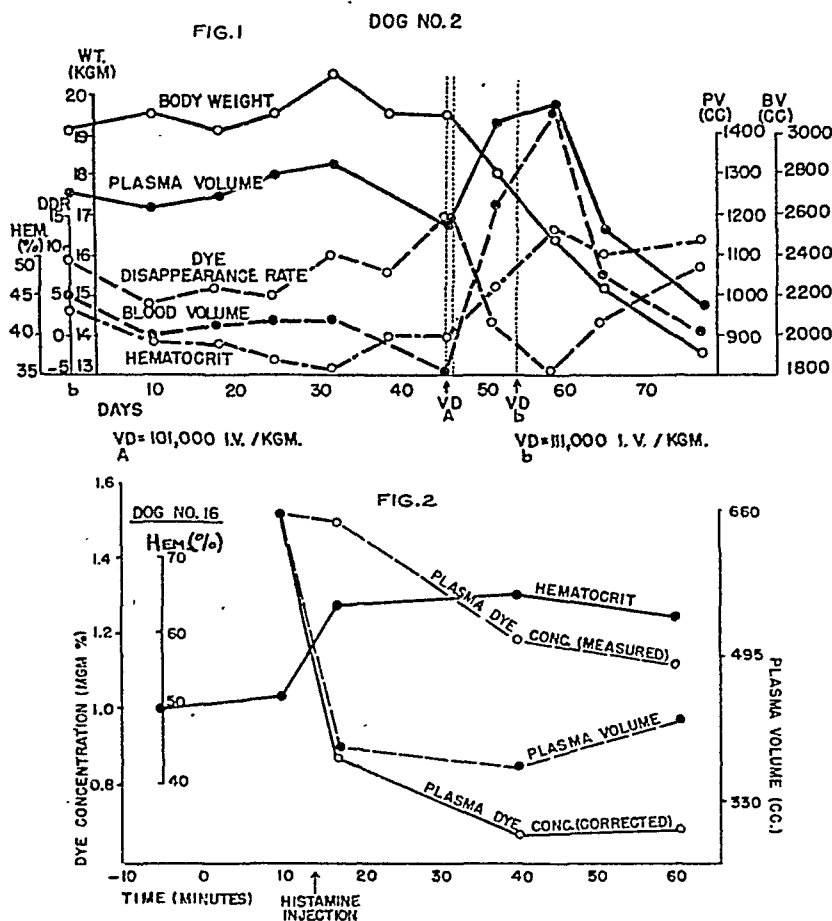


Fig. 1. TYPICAL EFFECT of concentrated dose of vitamin D (approximately 100,000 i.v./kg.).

Fig. 2. HISTAMINE EXPERIMENT on unanesthetized dog showing effect of plasma loss on plasma dye concentration. Correlations calculated according to text. Dye injected at 0 time.

body weight decreased in 28 out of the 30 cases (mean change was  $-11.5\%$ ). Figure 1 illustrates the findings on a typical animal.

In table 1 the data are arranged by degree of weight loss and it is apparent from inspection that the rise in hematocrit is related to the loss in weight (correlation coefficient,  $r$ , was found to be  $-.63$ ). The change in dye disappearance rate showed no correlation with weight loss, however ( $r$  was  $-.29$ ).

**Discussion.** The results indicated that a high dose of vitamin D produced decreased capillary permeability in dogs. This dose also caused anorexia and a decreased water intake on the part of most of the dogs, which was reflected in the loss of body weight over the experimental period. The consistent finding of the rise in

hematocrit associated with the loss in weight is in accord with the findings of DeBoer (11) on fasting, aquaprvic dogs, and probably indicates a compensatory activity of the spleen during a condition of impending dehydration.

The fact that there was a net decrease in plasma volume in most cases is again associated with the tendency to dehydration over the week's latent period. However, in 6 cases there was an exceptionally large increase in plasma volume and 5 of these occurred during extremely hot weather. One might postulate that a) the thirst mechanism overcame the anorexia in these cases, causing an influx of water into the blood from the gastrointestinal tract, and b) the condition of decreased capillary permeability prevented the simultaneous downward adjustment of this increased plasma volume, which would normally take place by the rapid movement of fluid out of capillaries in other regions. One might be permitted to speculate on the possible application of these findings to clinical conditions where it is desirable to bring about a stationary, moderately large blood volume. A large priming dose of vitamin D followed by the intravenous administration of nutrient fluids over the several days' latent period might achieve this end.

#### *Disappearance Rate of T-1824 as an Indicator of Capillary Permeability*

The validity of the method was investigated in two ways; first a study was made of the effect of a known capillary poison, histamine, on the dye disappearance rate, and, secondly, a quantitative study was made of the transfer of dye from the blood to the lymph in order to see if the rate of disappearance from the blood stream is a true indicator of the transfer of dye across the capillary barrier into the lymph.

*Effect of histamine on the dye disappearance rate.* Histamine was administered intravenously during the course of nine experiments. Six of these experiments were similar to the routine dye disappearance test and three were acute experiments involving the collection of lymph, as described in the next section.

It was found that the disappearance rate was increased several fold by histamine. The control values were 6 to 11 per cent for the six routine experiments, and 4 to 6 per cent for the three acute experiments. The disappearance rates found with histamine were 18 to 29 per cent for the former, and 12 to 19 per cent for the latter. Table 2 shows these data. Figures 2 and 3 illustrate curves of single typical experiments of the routine and acute types, respectively.

It should be pointed out that the disappearance rate measured during histamine administration, in all likelihood, does not fully reflect the true state. If we assume that histamine brings about a considerable exit of dyed fluid from the blood, as was evidenced here by the copious and heavily dyed lymph flow seen in the histamine-lymph experiments, then it is apparent that the dye disappearance rate as measured could not reflect the full degree of increased leakage. It is possible to make a partial correction for this by using the increased hematocrit as an index for the decrease in plasma volume, and thus to correct the measured dye concentrations by the factor by which the plasma volume was altered. This procedure, of course, neglects the factor of splenic contraction which may contribute to the rise in hematocrit, but nevertheless, the resulting 'corrected' disappearance rates are probably more accurate than the uncorrected ones. Table 2 includes the 'corrected' rates based on the hematocrit values, and these range from 29 to 54 per cent.

The entire group of 9 histamine experiments presented a mean value of 20 per cent uncorrected, and 40 per cent 'corrected' disappearance rates, to be compared with the control mean of 7 per cent (s.d. = 2.8%).

TABLE 2. EFFECT OF HISTAMINE ON DYE DISAPPEARANCE RATE

CASE	EXPERIMENTAL CONDITIONS	HEMATOCRIT 15 <sup>h</sup> -60 <sup>h</sup>	DYE DISAPPEAR. RATE	
Control Experiments				
15a	Routine determination, no anesthesia	% 51-50	% 11	
15b	" " " "	48-47	9	
15c	" " " "	48-48	6	
16a	" " " "	49-47	11	
16b	" " " "	51-50	7	
16c	" " " "	50-46	11	
20	Acute lymph experiment, anesthesia	43-45	5	
21	" " " "	38-41	4	
22	" " " "	55-57	6	
23	" " " "	51-53	5	
MEAN.....			7.5	
s.d.....			2.8	
Histamine Experiments				
			DYE DISAPPEAR. RATE	
			A <sup>1</sup> %	B <sup>1</sup> %
15d	Routine determination, no anesthesia	52-57	18	33
15e	" " " "	53-60	18	38
15f	" " " "	53-59	20	37
16d	" " " "	50-59	27	49
16e	" " " "	52-62	29	53
16f	" " " "	56-68	23	54
17	Acute lymph experiment, anesthesia	49-52	19	31
18	" " " "	59-63	16	29
19	" " " "	54-63	12	38
MEAN.....			20	40

<sup>1</sup> A = uncorrected; B = corrected according to text.

*Correlation of dye disappearance rate with transfer of dye from blood to lymph.* Ten fasted dogs were anesthetized with sodium barbital, and lymph from the thoracic or cervical lymph duct, which drained continuously, was collected for analysis during a control period and at two 15-minute periods which averaged 15 and 60 minutes after dye injection. Routine blood samples were taken simultaneously for determining the dye disappearance rate. In three cases histamine was injected during the



course of the experiment, and of the remaining 7 dogs two had received massive doses of vitamin D four months prior to the acute experiments.

The control dogs displayed a lymph dye concentration curve which rose very slowly over one hour to about one-fifth to one-third of the serum dye concentration. In the histamine experiments, the lymph became very blue and flowed copiously after the drug injection, reaching the plasma level of dye concentration by one hour (fig. 3).

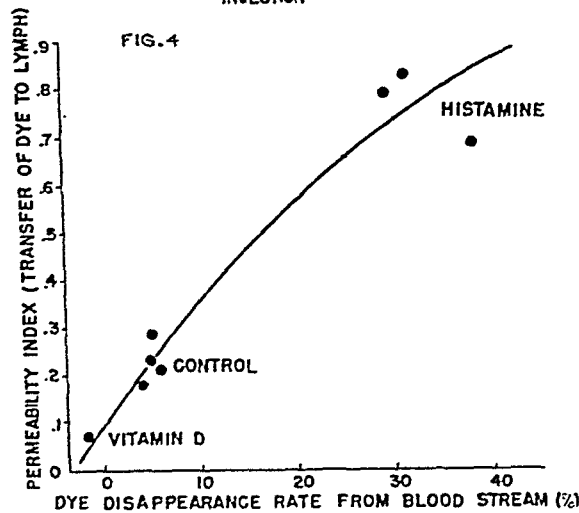
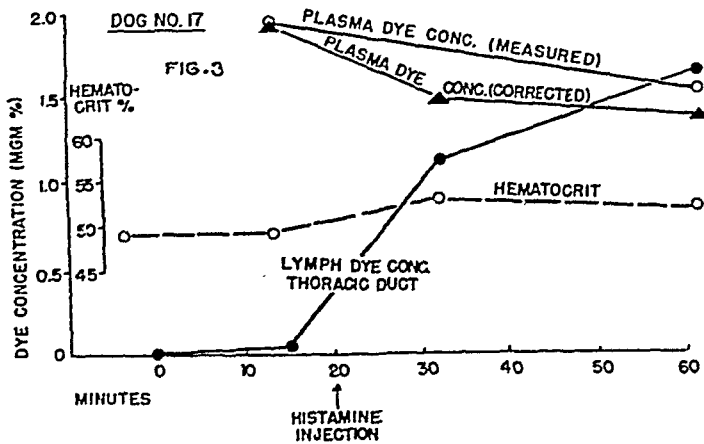


Fig. 3. ACUTE LYMPH EXPERIMENT on anesthetized dog showing effect of histamine on transfer of dye from blood to lymph. Dye injected at 0 time.

Fig. 4. Relation between dye disappearance rate and transfer of dye from blood to thoracic lymph.

In order to make a quantitative correlation between dye disappearance from the blood and dye transfer to the lymph, it was necessary to use some numerical index of dye transfer, which would take into account the varying values of original plasma dye concentration. The index used was the ratio of the increase in lymph dye concentration between 15 and 60 minutes after dye injection, over the 15-minute plasma dye concentration. This ratio was calculated for each of the 10 lymph experiments, and the resulting figures, termed 'permeability indices' are shown in table 3, grouped according to conditions of theoretically increasing capillary permeability. The dye

disappearance rates obtained simultaneously are also in this table. Two main points are brought out by these data. *a)* The figures for permeability index correspond qualitatively with the theoretical conditions of capillary permeability, from the decreasing effect of vitamin D, through the control range, to the increasing effect of histamine. *b)* In the case of the thoracic duct experiments, there is excellent correlation between the values for dye disappearance rate and the permeability indices obtained simultaneously. The correlation coefficient  $r$ , was found to be  $+0.956$ , indicating the validity of the dye disappearance rate test in detecting large differences in the conditions of permeability of the visceral capillaries. Figure 4 illustrates this point.

*Discussion.* The method used for testing capillary permeability seemed at first to be open to a number of theoretical objections. One was the rôle of phagocytosis

TABLE 3. PERMEABILITY INDEX UNDER VARIOUS EXPERIMENTAL CONDITIONS.  
CORRELATION WITH DYE DISAPPEARANCE RATE

CASE	LYMPH TESTED	EXPERIMENTAL CONDITIONS	PLASMA DYE CONCENTRATION		LYMPH DYE CONCENTRATION		PERMEABILITY INDEX $\frac{(D - C)}{A}$	DYE DISAPPEAR. RATE
			15' (A)	60' (B)	15' (C)	60' (D)		
			mg. %		mg. %			%
12	cervical	Vitamin D	1.20	1.08	0.00	0.00	.00	10
24	"	Control	1.93	1.75	0.00	0.08	.04	9
13	thoracic	Vitamin D	0.63	0.64	0.00	0.03	.05	-2
21	"	Control	4.00	3.85	0.07	0.78	.18	4
22	"	"	1.54	1.45	0.03	0.34	.21	6
20	"	"	3.30	3.15	0.17	0.94	.23	5
23	"	"	0.93	0.88	0.09	0.36	.29	5
19	"	Histamine	2.21	1.37	0.15	1.68	.69	38
18	"	"	0.91	0.65	0.12	0.84	.79	29
17	"	"	1.88	1.30	0.03	1.60	.83	31

as an independent variable. That this is of negligible importance is implied by the case of the dog described in the preliminary paper, where a large difference in the factor of phagocytosis is an independent variable. That this is of negligible importance is implied by the case of the dog described in the preliminary paper, where a large difference in the factor of phagocytosis (the reticulo-endothelial system was blocked) had no effect on the dye disappearance rate. This might be expected on the grounds that during the first hour after dye injection, the dye particles are mainly concerned with being distributed into the lymph.

Another objection was the effect of a possible contraction of the spleen during the course of a determination. However, since the spleen delivers very cell-rich blood, this would not greatly affect concentration of plasma constituents, and, furthermore, the rise in hematocrit would constitute a check on the test.

A change in the circulatory conditions of the liver might be expected to affect the

dye disappearance rate, and it is probably this factor which causes the considerable degree of control variation. These normal variations, however, are not large enough to invalidate the method for detecting marked changes in capillary permeability such as caused by histamine and vitamin D.

The strongest argument in favor of the method is the excellent correlation which was found between the dye disappearance rate from the blood stream and the measured transfer of dye from blood to lymph. It should be pointed out that the concentrations of plasma dye used here were low, and that the situation might be different with higher blood concentrations, a fact which was suggested by later work in which the amounts of injected dye were four times as great.

Naturally, the method would not be valid under extreme conditions, such as acute trauma, where the integrity of the vascular system might be so grossly impaired as to permit frank hemorrhage or leakage of large amounts of practically whole blood into extravascular spaces. Under these conditions, the concentration of any one element within the waning vascular pool could remain quite unchanged.

#### SUMMARY AND CONCLUSIONS

After a latent period of five to eight days following a highly concentrated dose of vitamin D, the following effects were observed in dogs: *a*) a decreased dye disappearance rate in 76 per cent of the cases; *b*) weight loss of approximately 10 per cent, caused by lowered intake of food and water; *c*) an increased hematocrit in 100 per cent of the cases. This phenomenon was ascribed to splenic activity accompanying the state of dehydration; *d*) a relatively unchanged plasma and total blood volume.

The administration of histamine caused the dye to disappear from the blood and appear in the lymph at a rate approximately four times greater than the controls. The quantitative study of the transfer of dye from blood to lymph over a wide range of conditions of capillary permeability, demonstrated an excellent correlation between the dye disappearance rate from the blood stream and the appearance of dye in the thoracic lymph.

A highly concentrated dose of vitamin D decreases the disappearance rate of T-1824 from the blood stream of dogs, after a latent period of about one week. The disappearance rate of the dye T-1824 from the blood stream, for low concentration of plasma dye, is a good indicator of the transfer of dye from blood to lymph, and thus, inferentially, measures the general state of permeability of the visceral capillaries.

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# SPECTROPHOTOMETRIC MEASUREMENT OF TRACES OF THE DYE T-1824 BY EXTRACTION WITH CELLOPHANE FROM BOTH BLOOD SERUM AND URINE OF NORMAL DOGS<sup>1</sup>

THOMAS H. ALLEN AND PETER D. ORAHOVATS

*From the Department of Physiology of the College of Physicians and Surgeons,  
Columbia University*

NEW YORK CITY

**D**URING the past 60 years a number of diazo dyes of biological interest have been synthesized by coupling o-tolidine with sulfonated aminonaphthols like 1-amino-8-naphthol-2,4-disulfonic acid (1). The latter product, briefly called T-1824, has become widely known because of its suitability for estimating plasma volume (2-9).

Several years ago it was shown that T-1824 has marked affinity for serum albumin, much lesser affinities for alpha and beta globulins and no affinity for gamma globulin (9) or fibrinogen (5). It is probable that T-1824 in the bloodstream exists chiefly in the form of a dye-albumin compound (7, 9). As previously recognized, the extraction of T-1824 from a dye-tinged blood sample involves the splitting of dye-albumin (10). There is a simple way of liberating dye from albumin and this can be done by adding a detergent such as Aerosol OT (11). Dye, thus liberated, also has an affinity for cellophane (9, 11). It therefore seemed possible that traces of T-1824 in various biological fluids could be both extracted and selectively concentrated on small foils of cellophane. Then the amount of dye could be estimated colorimetrically.

It is the purpose of this study to indicate the procedures which can be used in the quantitative estimation of T-1824 by a method based on these principles. The method has been tested by the recovery of known amounts of T-1824 in 0.2 ml. of blood serum and in half-hour urine samples. Small, definite quantities of this protein-binding dye were found to pass from the blood into the urine of dogs. The T-1824 clearance proceeds at the same rate as that predictable for serum albumin.

## GENERAL PROCEDURE

Two sizes of single thickness foils were cut from Cellophane dialyzer tubing.<sup>2</sup> A foil, about 1 x 2 cm. or 2 x 3 cm. in area, was suspended on a glass needle in order to hold the foil in a vertical position when immersed in fluids contained in a 50 ml. Erlenmeyer flask. The flask was covered with an inverted beaker and placed on a steam-coil water bath at 70° to 73°C. Test foils were soaked in saline, urine or diluted serum variously tinged with dye. Control foils were soaked in the same fluids except that dye was absent. The dyeing process was stopped at any desired point by transferring the foils to 0.9 per cent NaCl. Traces of adhering precipitate, when present, were removed by rinsing and if necessary by brushing.

Received for publication June 25, 1948.

<sup>1</sup> A preliminary report of this work appeared in *Federation Proc.* 7: 2, 1948.

<sup>2</sup> Purchased through Eimer and Amend, Inc. and listed as having a thickness of .000732 inches.

The optical density of the dyed foil in comparison to its control was determined with a König-Martens visual spectrophotometer. Both foils were wet with water and pressed between two microscope slides clipped against a slotted vertical brass plate. A foil, thus prepared, gave identical optical densities when shifted from place to place before the light beam. Also the readings did not change with time. Determinations made on a number of dyed foils showed that the maximum optical density occurred at  $635\text{ m}\mu$  whereas that (4) for dye in serum is at  $625\text{ m}\mu$  and in either 0.9 per cent NaCl or water at  $605\text{ m}\mu$ . In consequence the optical densities were always compared at the wave length of maximum absorption. Since the foils were not of equal area, although apparently of identical thickness, it next was necessary to know their dry weights in order to determine the total amount of dye present. The small foils ranged from 5.2 to 6.8 mg. and the larger from 14.7 to 18.1 mg.; they were weighed to the nearest 0.1 mg.

Known solutions of T-1824 in a given concentration of NaCl were prepared by diluting a standardized ampule solution (0.48 per cent in water) with glass distilled water and the required volume of 9.0 per cent NaCl.<sup>3</sup> Unless otherwise indicated the dilutions were made so as to give final NaCl concentrations of 0.9 weight per cent. All glassware used was Pyrex, cleaned with chromic acid, rinsed, and steamed.

### RESULTS

*Sorption equilibrium.* After 20 hours an equilibrium (fig. 1) occurred in the sorption of dye by a foil in the presence of 25 ml. of a  $1/10,000$  dilution of the ampuled dye. Equilibrium was indicated by the relative constancy of the *cellophane value*, which here is defined as the product of the optical density at  $635\text{ m}\mu$  and the weight in mg. of any foil. This dyeing process was quite striking to behold. The blue color of the fluid was very faint at the beginning, while at the end the foil was blue and the fluid was colorless. If all the dye had migrated to the foil, this would mean that more than a 5000 fold concentration step occurs in the transfer from the original dispersion volume.

In order to test for completion of sorption the foils were removed after 23 hours from three reaction flasks and a new foil was placed in each flask. Twenty-four hours later these second cellophane values were less than two per cent of the first ones. When for three other reactions the first 18-hour cellophane values were compared with their second values, the latter were less than six per cent of the former. These results together with those obtained from a  $2/10,000$  dilution, lead to the conclusion that at equilibrium at least 98 per cent of the dye combines with the foil.

*Influence of electrolyte and volume on sorption changes.* NaCl had a marked effect upon the equilibrium position. Sorption was nearly complete in 0.9 to 3.0 per cent NaCl, whereas it was incomplete in lesser concentrations of NaCl (fig. 1, insert). As previously observed (9) there apparently was no sorption from aqueous solutions. Water also removed dye previously sorbed from NaCl solutions. These facts were ascertained by the following additional means. A dyed foil with a cellophane value of 2.48 together with an undyed foil was placed in 25 ml. of glass distilled water at  $70^\circ\text{C}$ . Eighteen hours later both foils were colorless and the water had become tinged with blue.

The pH was changed in two separate dilutions of the same amount of dye by making each dilution 0.01 M with respect to either monobasic or dibasic phosphate buffer salt. The glass electrode readings corresponded to pH 4.6 and to pH 8.5.

<sup>3</sup> The blue dye T-1824 was furnished by the Warner Institute for Therapeutic Research, New York City.

Triplicate cellophane values from these two solutions were identical with those from an unbuffered solution of the dye.

At equilibrium the cellophane values remained unchanged whether the volume was 25 ml. or had been increased by the addition of 75 ml. of 0.9 per cent NaCl. Therefore, it is concluded that sorption at 70°C. is relatively uninfluenced by these changes in pH or volume, provided the NaCl concentration is at least 0.9 per cent.

*Foil size, cellophane value and amount of T-1824.* A small foil (5 to 7 mg.) was placed in 25 ml. of any one of six different concentrations of T-1824. After equilibrium time at 70°C. the cellophane value was estimated. The means for triplicate or quadruplicate determinations, thus obtained, are plotted in figure 2. It may be seen that in less than 2/10,000 dilutions of ampuled dye a direct proportionality was found between the amount of dye originally in solution and the cellophane value

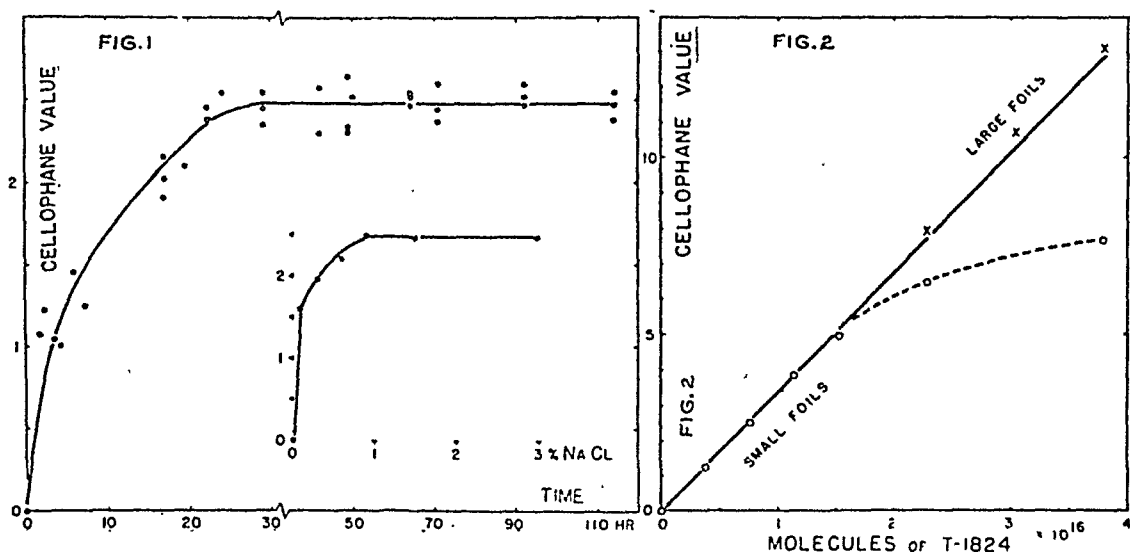


Fig. 1. SORPTION OF T-1824 DYE by cellophane as a function of time from 25 ml. of 1/10,000 dilution of 0.48 % T-1824 in 0.9 wt. % NaCl. Temperature is 70° to 73°C. Insert: promotion of sorption equilibrium by NaCl. Both ordinates express units of cellophane value = optical density at 635 mμ × wt. mg. of foil 0.00186 cm thick.

Fig. 2. RELATION BETWEEN FOIL AREA, cellophane value and amount of T-1824. Dilution range is 0 to 5 parts in 10,000 of 0.48 % T-1824 in 0.9 wt. % NaCl.

developed at equilibrium. Beyond this concentration the direct proportionality ceased to exist as indicated by the departure from linearity (dotted line, fig. 2), at 3/10,000 and 5/10,000 dilutions and also by the visible retention of dye in the latter solution.

When larger foils (15 to 18 mg.) were used the cellophane values from 3/10,000, 4/10,000 and 5/10,000 dilutions of T-1824 returned to the same proportionality obtained for the small foils in the lower concentrations of dye (solid line, fig. 2.). Evidently an excess of cellophane must be present in order to demonstrate a simple, linear relationship between the amount of dye and the cellophane value. In 25 determinations the two greatest deviations from proportionality were less than ±8 per cent. Since unknown variations in dyeing and known variations in instrumenta-

tion could account for the observed deviation, it is concluded that this direct relationship is an index of the amount of T-1824.

It is possible to relate the cellophane value of a dyed foil to the number of molecules of T-1824. If in these solutions the dye is a monomer, the molecular weight is 960 (1) and the calculated number of molecules in 25 ml. of a 1/10,000 dilution is  $0.76 \times 10^{16}$ . Thus, according to the data of figure 2, a cellophane value of 1.00 represents  $0.30 \times 10^{16}$  molecules of T-1824.

The above cellophane values for T-1824 were obtained with foils cut from the same piece of cellophane. This material was 0.00186 cm. in thickness. For the sake of future comparison with foils of a different thickness but identical chemical constitution, it is necessary to state the molar absorption coefficient,  $\epsilon$ , for the dye cello-

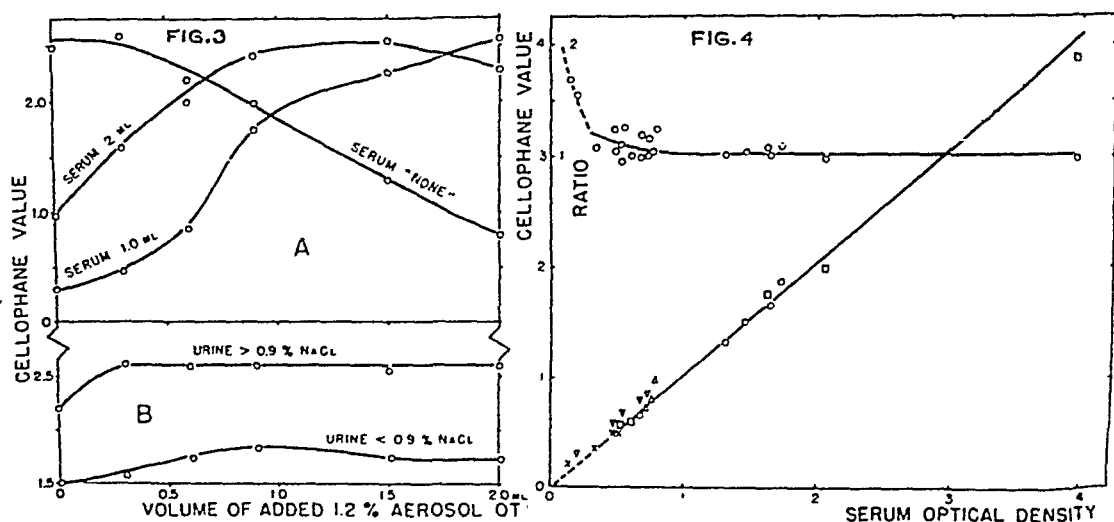


FIG. 3. EFFECT OF AEROSOL OT on sorption and recovery of T-1824 from dog blood serum and urine. A cellophane value of 2.50 corresponds to recovery of the  $7.6 \times 10^{15}$  molecules or 12.2  $\mu\text{g}$ . of T-1824 originally dispersed in the diluted blood serum or urine. A: when 0, 0.2 or 1.0 ml. of blood serum is first mixed with 25 ml. of 1/10,000 dilution of 0.48 % T-1824 in 0.9 wt. % NaCl. B: when NaCl is either added or not added to 25 ml. of 1/10,000 dilution of 0.48 per cent T-1824 in urine of water diuresis.

Fig. 4. COMPARISON OF CELLOPHANE VALUE with serum optical density (in 0.5 cm. cuvettes) for successive blood samples from 5 dogs.

phane. This coefficient was calculated from the standard equation,  $\epsilon = D/c.d$ , expressing both dispersion volume and thickness in terms of mass and specific gravity. The latter cancels out and the final equation becomes:  $\epsilon = K \cdot DM/N$ , where  $DM$  is the cellophane value,  $N$  the number of molecules in the solution and  $K$ , a constant depending on foil thickness and equilibrium position, is  $1.99 \times 10^{20}$ . Fifty-one determinations, illustrated in figures 1 and 2, yield a mean of 66,300 and standard error of  $\pm 3,700$ . This coefficient is characteristic of dye-cellophane. It is smaller than that of dye-albumin. The molar absorption coefficient of T-1824<sup>4</sup> in either blood plasma or serum is 77,800. This value increases in 0.9 per cent NaCl to 85,200 and in water to 93,800.

<sup>4</sup> The following values for the Fieser preparation (1, 4) of T-1824 were calculated from the original data (4), kindly furnished by M. I. Gregersen. For this and other favors including the criticism of this manuscript the authors wish to express their gratitude.

*Liberation of dye from diluted serum by Aerosol OT.*<sup>5</sup> When blood serum containing 5.4 per cent protein (refractometric analysis) was mixed with solutions of T-1824, less dye was available for sorption by cellophane. This effect was qualitatively observed by Rawson (9) and was quantitatively verified at a higher temperature in the course of the present study. The values on the ordinate of figure 3a show that the addition of 0.2 ml. of serum to 25 ml. of a 1/10,000 dilution of 0.48 per cent T-1824 reduced the cellophane value from 2.42 to 0.96 and with 1.0 ml. of serum the value fell to 0.30. Dye was not sorbed from undiluted serum.

Aerosol OT altered the foregoing relationship among the components of this dye-protein-cellophane system (11). When sufficient Aerosol OT was added at room temperature to either the mixture of dye and serum or to only the serum solution, a faint turbidity immediately appeared. However, there was no turbidity in the absence of serum. The Aerosol OT evidently freed dye from protein as indicated by the upward course of the two serum curves in figure 3a. When 0.9 ml. of the Aerosol OT solution was added to the mixture of 25 ml. of T-1824 solution and 0.2 ml. of serum, an equilibrium cellophane value of 2.38 was attained. Approximately all of the  $7.6 \times 10^{15}$  molecules of T-1824 were therefore combining with the cellophane. The data also show that a greater amount of Aerosol OT was needed to free the dye from 1.0 ml. of serum. In the absence of serum (fig. 3a, serum none) there was a decrease in sorption of dye with the addition of Aerosol OT. Whether in the latter system the Aerosol OT competes for T-1824 or for cellophane is unknown. However, the arrangement of this family of curves (fig. 3a) provides a means of testing for protein. The rising curve shows the presence, whereas the falling curve must indicate the absence of dye-binding protein.

Previous observations together with those noted above suggest that this liberation of dye from serum proceeds through complex reaction steps involving several features of interest. Reagents, such as trichloroacetic acid, precipitate the usual dye-tinged serum leaving all of the dye still combined with the insoluble proteins (6). This sort of combination has recently been measured in various mixtures of T-1824 and serum albumin or globulins (12) at pH 2.5. In contrast to the usual protein precipitants various synthetic detergents have properties of interest to recovery of T-1824 from serum proteins. There are many known reactions between various proteins and polar-nonpolar anions of different chemical structure containing more than 10 carbon atoms. Aerosol OT in sufficient amount was early found to denature beef hemoglobin (13). Mixtures of horse serum albumin and sodium dodecyl sulfate combine in characteristic fashions in relation to pH and temperature (14). It is therefore probable that Aerosol OT reacts with the serum proteins and in so doing combines with at least those groups available for attachment of T-1824, thus freeing dye for sorption on cellophane. This competition between Aerosol OT and T-1824 is similar to that observed by Klotz in which a number of different organic anions compete with sulfonated anions such as azosulfathiazole for the  $\epsilon$ -ammonium of lysine in bovine serum albumin (15).

*Estimation of dye in blood serum.* Dye-tinged serum was obtained from 5 dogs none of which had ever received T-1824. A control blood sample was taken. Then

<sup>5</sup> The dioctyl sodium sulfosuccinate described in Aerosol Wetting Agents, 1946, American Cyanamid Co.



a measured volume of 0.48 per cent T-1824 solution was injected into a jugular vein. After 15 minutes and at intervals during the next four to six hours, dye-tinged blood was withdrawn from the opposite jugular vein. A blood sample was also taken on the following day.

Direct spectrophotometric determination of optical density at  $624\text{ m}\mu$  was made on each of these 22 samples using 0.5 cm. cuvettes. One of the samples was so dense that it was necessary to make a two-fold dilution with 0.9 per cent NaCl in order to measure its optical density.

Triplicate cellophane determinations were also made on each of the samples. To 25 ml. of 0.9 per cent NaCl was added 0.2 ml. of dye-tinged serum and 1.0 ml. of Aerosol OT solution (1.2 per cent in water). After one-half hour at room temperature a small foil was added, and the flask was covered and placed at  $70^{\circ}\text{C}$ . for 24 to 30 hours. A control foil was similarly exposed to the serum obtained before injection of dye. The means for these cellophane values are indicated along the ordinate in figure 4.

Since the reaction between dye-protein and Aerosol OT releases all of the dye (fig. 3a), it was not surprising to have found the linear relationship which intersects the origin in figure 4. When the serum was deeply tinged with dye, the cellophane value from 0.2 ml. was only 1.02 times greater than the serum optical density. With serum optical densities between 1.0 and 0.3, the cellophane value increased to 1.10 times as indicated by the slight upward trend of the ratio curve (fig. 4). Two of the 24-hour samples, which were less than 0.3 optical densities, gave unusually high cellophane values thus causing the ratio to approach 1.7:1.0 and perhaps marking a lower limit where instrumental error makes it difficult to secure accuracy with only 0.2 ml. of serum. The near identity of the cellophane value and the serum optical density occurred only through the chance that the chosen volume of serum contained the quantity of dye which gave these cellophane values on foils of this particular thickness. For example, doubling either the foil thickness or the volume of dye-tinged serum yielded cellophane values which were approximately twice the values for directly measured serum optical densities.

*Recovery of T-1824 from urine.* A solution was prepared by diluting 25 ml. of a 1/1,000 dilution of 0.48 per cent T-1824 to 250 ml. with dog urine. The urine had been obtained by catheter in the course of a diuresis following the introduction by stomach tube of tap water in the amount 80 ml/kg. of body weight.

The equilibrium cellophane value from 25 ml. of this solution of dye in urine was 1.50 (fig. 3b) and was never greater than 1.85 when Aerosol OT was added. However, the addition of 2.5 ml. of 9 per cent NaCl together with Aerosol OT yielded cellophane values as great as 2.60, which represents complete recovery of dye. The dyeing process was promoted by NaCl but at least 0.3 ml. of 1.2 per cent Aerosol OT was also needed for complete sorption. This, together with the fact that sorption did not decrease with more Aerosol OT, showed that there was binding of both T-1824 and Aerosol OT by urine constituents.

*Excretion of T-1824 in the urine.* A typical experiment was performed using a 12.5 kg. dog which had never received any T-1824. Tap water, 0.7 l., was introduced by stomach tube. A urinary catheter was inserted, and an one-hour urine sample

together with the usual 10 ml. of 0.9 per cent NaCl rinse was collected before the taking of control blood and the injecting of 6.5 ml. of 0.48 per cent T-1824. During the next five hours urine was collected at half-hour periods. Then the animal was turned loose. Shortly before the eighth hour 0.3 l. of water was given and an one hour urine sample was obtained. This routine was repeated for the 12th-, 24th-, and 75th-hour urine samples. Blood samples were taken at different times. The time-concentration curve of T-1824 in the plasma (5) is shown in figure 5.

To each of the urine samples, thus collected, was added one ninth its volume of 9 per cent NaCl, 1.0 ml. of 1.2 per cent Aerosol OT and one of the small-size cellophane foils. After 30 hours at 70°C. the foil was removed, and its cellophane value

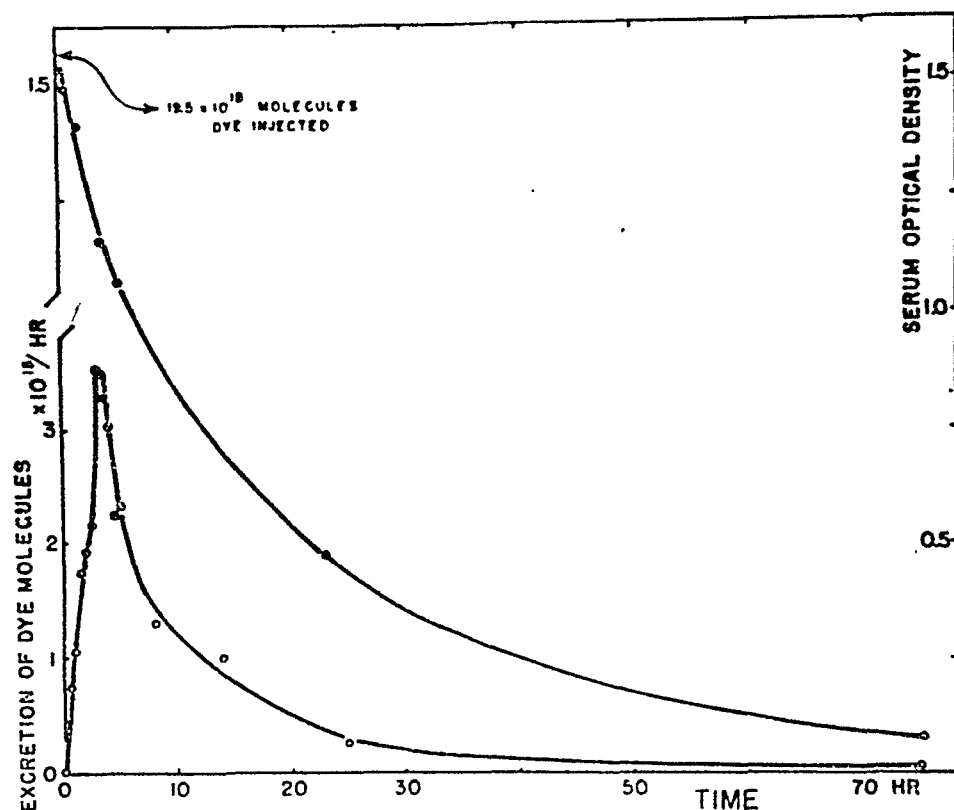


Fig. 5. RATE OF EXCRETION and serum optical density of T-1824 (in 0.5 cm. cuvettes).

was estimated from the optical density and weight. The amount of T-1824 in any urine sample was found by the correspondence of unit cellophane value to  $3.0 \times 10^{15}$  molecules of T-1824. The hourly rates of excretion are indicated by the lower curve, which rises for  $3\frac{1}{2}$  hours and then falls (fig. 5).

Very little dye was excreted (table 1). In the first day only 0.15 per cent of the amount injected appeared in the urine. This value fell during the next two days to only 0.04 per cent. Most of the dye which disappeared from the blood obviously left by routes or mechanisms other than the kidneys (7, 12, 16). Nevertheless, renal excretion of dye was related to the plasma concentration. At any moment the amount gained by the urine was about 0.2 per cent of the amount lost from the circulation. Although the plasma concentration decreased to 30 per cent (fig. 5), the

renal clearance of T-1824 remained at 1.4 or 1.2  $\mu$ l. per minute throughout the first day following injection of dye.

At the concentrations used for estimation of plasma volume, T-1824 has not previously been found in urine unless abnormal amounts of protein were also excreted (2, 7, 17). The cellophane test shows that dye is also excreted by the normal kidney but in such minute amounts that the visible presence of dye is masked by the usual color of urine. Since preliminary qualitative tests on 6 dogs showed that dye was excreted following the intravenous injection of T-1824, quantitative studies were made on 8 additional dogs, 4 of which had water diuresis. The results show that similar renal clearances occurred irrespective of sex, weight or urine flow (table 2).

TABLE 1. EXCRETION OF T-1824 BY THE KIDNEY DURING PERIODIC WATER DIURESIS IN DOG 8 INITIAL PLASMA VOLUME IS 610 ML.

TIME AFTER INJECTION	MOLECULES T-1824 EXCRETED $\times 10^{-15}$	PERCENTAGE OF AMOUNT INJECTED	PERCENTAGE OF AMOUNT LOST FROM PLASMA	RENAL CLEARANCE
hr.				$\mu$ l/min.
0.5-3.5	7.0	0.04	0.18	1.4
3.5-24	21.3	0.11	0.24	1.2
24-75	7.9	0.04	0.18	0.7

TABLE 2. RENAL CLEARANCE OF T-1824 IN INDIVIDUAL DOGS ARRANGED IN ORDER OF AVERAGE URINE FLOW

DOG	SEX	WT.	TIME AFTER INJECTION	URINE FLOW	CLEARANCE
		kg.	hr.	ml/min.	$\mu$ l/min.
1	Female	10.2	0.5-4.5	0.04	1.7
2	Female	9.4	0-8	0.05	2.0
3	Male	9.0	0.5-8	0.08	1.9
4	Male	10.9	1-6	0.09	1.4
5	Female	11.0	0.5-4.5	0.08	2.2
6	Male	13.0	0-6	1.03	2.1
7	Male	10.5	0-6.5	1.04	1.4
8	Male	12.5	0.5-3.5	1.37	1.4

Höber's studies offer reasons for suspecting that T-1824 is not secreted. Since disulfonated dyes with symmetrically distributed sulfonic acid groups are not actively transferred from solution in the Ringer-perfused renal portal system of a frog (18), it might be inferred that the tetrasulfonated T-1824 dye would have escaped only by passive transfer. This inference together with the observed clearances suggests that T-1824 passes into capsular fluid in the course of glomerular filtration. After being filtered, the amount of T-1824 collected in the urine is independent of variations in tubular reabsorption of water.

T-1824 in the amounts injected for determination of plasma volume probably is present in the blood stream only as dye-albumin (7, 9). Hence, it was decided to compare the T-1824 clearance with that for protein calculated from data found by a sensitive physical method. The clearance of T-1824 and of albumin should be

identical. The surface film area technique has recently given evidence for a mean concentration of 3.7 mg. per cent of protein in urine of normal human males (19). Gunton and Burton do not speculate on the route of protein excretion, but if the urine protein of their subjects had been albumin, the plasma albumin 3.5 per cent, and urine flow one ml. per minute, then the albumin clearance should be one  $\mu$ l. per minute. This clearance is of the same order of magnitude as that for T-1824 in the dog.

#### DISCUSSION

A method has been devised for the extraction and estimation of amounts of T-1824 as small as 0.5  $\mu$ g. The dye is sorbed on a cellophane foil and measured with a spectrophotometer. The principle of the method could be described as being an extension of the Detergency Triangle of McBain (20). The extension to the T-1824 system may ideally be given by the following equation:

$$\text{protein} \cdot \text{dye} + \text{soap} + \text{cellophane} = \text{protein} \cdot \text{soap} + \text{cellophane} \cdot \text{dye}.$$

Although there is a strong affinity of dye for protein, advantage may be taken of the affinity of T-1824 for cellulose in order to extract this dye from blood serum or urine. The amount of soap needed for the desired ion exchange was found by mixing various amounts of Aerosol OT with protein·dye and testing for sorption of dye on excess cellophane. With cellophane foils of identical area and thickness the optical density at 635  $m\mu$  alone was an index of dye sorption. Since it was neither convenient nor accurate to cut all of the foils to the same area, the T-1824 was measured by the product of the optical density and the dry weight of a foil which was 0.00186 cm. thick. This product is called the cellophane value for T-1824 and is proportional to the amount of dye present in the original dispersion volume. For example, a cellophane value of 1.00 corresponds to  $3.0 \times 10^{15}$  molecules or 4.8  $\mu$ g. of T-1824.

Many of the factors which influence the sorption of T-1824 are recognized to be of importance to the physical chemistry of dyeing. A survey of this subject (21) lists dyes which are similar in their behavior to that of T-1824. The characteristics of various textile dyes have been quantitatively studied by means of their sorption on regenerated cellulose films. Diffusion within cellulose itself is believed to be the slowest reaction step encountered in the entire dyeing process. Finally, through comparison with the dyes observed by Valko (21), it could be expected that the anionic dye T-1824 would be dispersed as a monomeric anion in 0.9 per cent NaCl at 40° or 70°C. In this connection the present report describes studies on the sorption of T-1824 as affected by temperature, dye concentration, NaCl, pH, Aerosol OT and serum or urine constituents. Consequently, the conditions may be stated under which dye can be extracted from blood or urine as completely as it can be from NaCl solutions. In the presence of excess cellophane the sorption of T-1824 is at least 98 per cent complete after more than 20 hours at 70°C. in NaCl of 0.9 to 3.0 per cent. Sorption is unaffected by changes in dispersion volume from 25 to 100 ml. and in pH from 4.6 to 8.5. However, sorption does depend on the availability of free T-1824 and hence on the absence of free serum protein or excess of free Aerosol OT. If 1.0 ml. of 1.2 per cent Aerosol OT is added to a mixture of 0.2 ml. of dye-tinged dog blood serum and 25 ml. of 0.9 per cent NaCl maximum sorption occurs. The fact that Aerosol OT was needed for complete liberation of T-1824 from diluted serum (figs.

3a and 4) lends support to the view that this dye is strongly bound to albumin (7, 9) and that the relatively slow disappearance of T-1824 from the bloodstream is a function of its marked affinity for serum albumin (9). It is necessary to make dog urine at least 0.9 per cent with respect to NaCl and to add at least 0.3 ml. of the Aerosol OT solution in order to get maximum sorption from half-hour urine samples.

In the course of the present studies it was noted that there was no change in the optical density at  $635\text{ m}\mu$  of the control foils. Evidently the exposure to dye-free urine or blood serum did not result in sorption of urine or serum constituents with chromophoric groups like those of T-1824. This observation indicates that further work may discover a practical advantage in use of this cellophane method to overcome the difficulties encountered (8) when lipemia or varying hemolysis is present, both of which interfere with direct spectrophotometric estimation of T-1824 in the serial blood samples used for measurement of plasma volume. Since the cellophane value of T-1824 from 0.2 ml. of serum is only 1.10 to 1.02 times greater than the serum optical density, it can be corrected and substituted into the equation (8) used in calculating the plasma volume.

The rate of arrival of T-1824 in the urine with respect to the T-1824 concentration in dog blood yields a plasma clearance of 1 to 2  $\mu\text{l.}$  per minute. By assuming this clearance value to be entirely a function of renal activity, it then becomes of interest to inquire into whether T-1824 leaves the blood in the form of free dye, as a dye-albumin compound or as a mixture of the former and latter. This inquiry will consider the evidence for the binding of dye in blood serum and also in urine. For the purpose of measuring the plasma volume the initial plasma concentration of T-1824 is approximately 0.002 per cent (8). As revealed by electrophoretic analysis a 0.004 per cent concentration of dye in four-times diluted blood serum at pH 7.4 is entirely bound by serum albumin (9). If the dye concentration is increased to 0.098 per cent it then combines with alpha and beta globulins as well as with albumin. It is known that ionized basic groups in one mole of albumin bind somewhat less than 14 moles of T-1824 (9), but the dissociation constant for the dye-albumin at pH 7.4 is unknown. LeVeen and Fishman find that T-1824-albumin at pH 2.5 has an apparent dissociation constant of  $K = 2 \times 10^{-6}$  and that at this low pH one mole of albumin binds at least 70 moles of dye (12).<sup>6</sup> There are several reasons for believing that  $K$  is less than  $2 \times 10^{-6}$ : it has been calculated for molarities existing before dilution and precipitation with trichloroacetic acid solution; competition between anions of trichloroacetic acid and T-1824 has not been considered; at the low pH the sulfonic acid groups of T-1824 are not entirely ionized (10), i.e. their  $\text{p}K \gg 1$  and  $< 2.5$ . For lack of information it is impossible to discuss the effect of electrostatic repulsion between T-1824 anions and T-1824-albumin as has been done in both fact and theory for azosulfathiazole (22). However, the value  $K = 2 \times 10^{-6}$  suggests that T-1824 has an affinity for serum albumin which is several hundred times that of azosulfathiazole. The actual dye-albumin formed in blood is probably so weakly dissociated that the arrival of dye in the urine is an index of albumin excretion. Urine albumin is very dilute and must certainly be affected by urine constituents, yet it was necessary

<sup>6</sup> In reply to a question in a letter from one of the authors a letter from H. H. LeVeen in part indicates that their published value is in error, should be changed from  $2 \times 10^{-3}$  to  $2 \times 10^{-6}$ , and this change will be requested of the editors.

to add Aerosol OT to dog urine in order to liberate dye for more complete extraction by cellophane.

#### SUMMARY AND CONCLUSIONS

The blue toluidine dye T-1824 has been quantitatively extracted from 0.9 to 3.0 per cent NaCl, blood serum and urine. Complete extraction, involving a great concentration step, is approached in the presence of excess cellophane after 24 hours at 70°C. Sorption is independent of certain pH or dispersion volume changes. The resulting dye-cellophane compound has a maximum light absorption at 635 m $\mu$  where the molar absorption coefficient is 66,300. The amount of sorbed dye was estimated in terms of the product of the optical densities at 635 m $\mu$  and the dry weights in mg. of foils which were .00186 cm. thick. This product, called the cellophane value for T-1824, at unity corresponds to  $3.0 \times 10^{15}$  molecules or 4.8  $\mu$ g. of T-1824. Amounts as small as 0.5  $\mu$ g. of T-1824 have been estimated.

Twenty-six determinations with a mean cellophane value of 2.50 had a standard error of 0.14.

In order to liberate dye for sorption by cellophane it is necessary to add the anionic detergent, Aerosol OT, both to diluted blood serum and to urine.

At the dye concentrations used for estimation of plasma volume the cellophane value with foils of this thickness for T-1824 from 0.2 ml. of dog blood serum is nearly equal to the serum optical density at 624 m $\mu$  in 0.5 cm. cuvettes.

A discussion of the occurrence of T-1824 in dog urine following its intravenous injection is presented with respect to the possibility that the T-1824 and naturally occurring albumin clearances are identical.

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# SHOCK DUE TO ELECTRICAL INJURY IN FROGS

LOUIS MOREAU,<sup>1</sup> MARVIN BALISTOCKY AND L. V. HEILBRUNN

*From the Zoological Laboratory of the University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

IN THE PAST, study of the complex and confusing problems of shock have been left almost entirely to the clinical physiologist and the pathologist, and yet, apparently, there are aspects of the problem which can be attacked from the standpoint of general or cellular physiology. This work is part of a larger plan to study the pathogenesis of injury shock from the standpoint of cellular physiology.

If shock is produced largely as a result of the formation of toxic substance by injured tissues (and this is now generally admitted by those who follow the literature), then we should seek to discover why and how the injured cells produce toxic substances, what sort of substances are involved, and in what manner they produce their effect on cells in other parts of the animal.

For many years, one of us (Heilbrunn) has maintained that when cells are stimulated or injured, a protoplasmic change similar to blood clotting occurs (1, 2). It was postulated that this protoplasmic clotting produced a thrombin-like (or, perhaps, a thromboplastic) substance which could induce clotting in cells distant from those injured. When the protoplasm of a muscle is clotted, this results in contraction (3). Hence, one could easily assume that injured cells give off substances which would pass through the circulation to smooth muscle cells at some distance and that these cells would undergo violent contraction. The evidence in favor of such a point of view is abundant and many-sided, and some of it has been summarized in an earlier paper (4).

The purpose of this paper is to further test the above hypothesis. If injury to tissues produces injury substances which have an effect on protoplasmic clotting, presumably these same substances would also affect the clotting time of the blood. Accordingly, we attempted to produce injury and then study blood-clotting times in the injured animal.

## MATERIAL AND METHODS

Frogs, *Rana pipiens*, weighing 17 to 23 gm. and of both sexes, were chosen as the experimental animal. In all, over 240 animals were used (including 40 controls).

Electrical stimulation was provided with an Ene-Volt, a variable transformer type of stimulating apparatus manufactured by Gorrell and Gorrell. Electrodes used in the muscle experiments were either copper wires or small iron plates measuring 1 x 2 cm. The current used had an E.M.F. varying between 10 and 400 volts and was applied for periods of one second to 20 minutes. The best results were obtained by using four pairs of plate electrodes fastened to the legs so that the thigh and calf

Received for publication June 3, 1948.

<sup>1</sup> National Institute of Health Fellow.

of each leg would be shocked simultaneously. An E.M.F. of 300 volts was used with this method, and shocks of one-second duration were given one or two times per minute. The current intensity with this method was 800 milliamperes through the entire system. The maximum temperature developed, measured by a fine wire thermocouple, was 33°C. in the thigh and 34°C. in the calf of the leg.

In the brain injury experiments, the electrodes were made of narrow sheet-iron strips painted with Cenco cement (a modified de Kotinsky cement), which is a non-conductor. The contact areas were 2.5 x 3.0 mm. One electrode was placed in the midline of the skull, over a spot between the orbits and the auditory plaques. The other electrode was placed in the midline of the roof of the mouth immediately anterior to the downward bulging of the orbits. The best results were obtained by using an E.M.F. of 100 volts and giving 10 shocks of one-second duration at the rate of one shock per minute. The current intensity delivered was 160 milliamperes. The maximum temperature reached was 34°C.

The blood-clotting time was determined by the capillary method. The heart was exposed through an opening in the upper abdomen and lower thorax. Blood samples were obtained by puncturing the ventricle with a capillary tube drawn out to a diameter of approximately .25 mm. The small end was cut off by scratching with a sharp piece of carborundum. This is an important detail because the end thus cut penetrates readily into the ventricle, promoting speed and minimizing the chance of error. Contamination of the blood sample by pericardial or tissue fluids produces error by shortening the clotting time. This was avoided by cutting away the anterior portion of the pericardial sac and blotting the heart with filter paper. We followed as a standard rule: never to take a blood sample from a visibly moist ventricle. Care was taken, as far as possible, to make each puncture through a fresh, unbroken portion of the ventricle. In the serial determination two samples were taken in immediate succession and the average value determined for each pair. In both the experimental and control animals used in serial determination, the relations of blood loss due to sampling were as follows: in each individual puncture approximately .0050 to .010 cc. of blood was withdrawn. The average amount lost through bleeding at each puncture was .010 cc. Most of the animals were sampled six to eight times (12-16 punctures) and thus had a total blood loss of 0.21 to 0.28 cc., or 16 to 20 per cent of the estimated total blood volume. The animals were neither pithed nor anesthetized.

#### OBSERVATIONS

*Local and distant effects.* In the muscle injury experiments the lower current intensities and brief shocking procedures produced only a temporary coma within 45 to 90 minutes after injury. The higher dosages produced irreversible effects. With the copper wire electrodes an E.M.F. of 75 volts for 4 minutes produced irreversible depression and coma in 30 to 60 minutes.

When a single pair of plate electrodes was used, death was caused by an E.M.F. of 200 volts applied for 7 seconds to each thigh and calf, as follows: 3 shocks of two seconds duration spaced approximately one minute apart followed by a 4th shock of one-second duration. When four pairs of electrodes were used, shocking all parts of the legs simultaneously, the E.M.F. was 300 volts.

The local effects were characterized by *hyperemia* of the skin beneath and be-



tween the electrodes and *marked swelling and rigor* of the hind legs, at first between the opposite electrodes. Later the swelling involved the entire legs, and the rigor became somewhat relaxed.

The general effects consisted of a slight lethargy, which disappeared in 3 to 5 minutes after injury. In about 20 minutes lethargy returned; and sooner or later, stupor developed. The front legs became sluggish. With this there was an apparent loss of initiative, which was accompanied by loss of the corneal reflex. During this stage the animal was still responsive to mechanical stimuli. In about 30 minutes to one hour came the stage of marked depression, during which no movement at all or only the very slightest movement of the front legs could be elicited by prodding.

The effects on the heart varied in degree. In the experiments with wire electrodes the heart continued to beat for 12 to 24 hours after the onset of coma. In the other experiments with plate electrodes, the heart stopped usually within  $2\frac{1}{2}$  to  $3\frac{1}{2}$  hours after the onset of depression.

TABLE 1. EFFECTS OF ALTERNATE REMOVAL AND REAPPLICATION OF LIGATURES TO THE LEGS OF A FROG GIVEN ELECTRICAL INJURY

TIME	PROCEDURE	REACTION
min.		
0	3 female frogs shocked on each thigh and calf of the leg, then ligatures were applied just below the inguinal region	All frogs remained briskly reactive and normal, except for loss of use of hind legs
60	Left leg of 1 frog freed of ligature	Depression observed within 10 minutes. Loss of use of front legs
85	Left leg retied	Within 20 minutes brisk reactivity observed
150	Right leg freed of ligature	Depression returned within 10 minutes
165	Right leg retied	Within 10 minutes, some return of reactivity

The 2 remaining frogs (with ligatured legs) remained briskly reactive for at least 6 hours.

In brain injury the toxic effects were variable. Coma beginning with the first shock and lasting from 15 minutes to  $2\frac{1}{2}$  hours was observed in nearly all the animals. The animals which recovered lived 16 hours to three days. The animals receiving 80 or 100 volts had irreversible coma.

*Effects of interrupting the circulation.* The circulation was interrupted either by excision of the heart or ligating the thighs immediately before inflicting injury. In all cases the depressant effects of electrical injury were confined to the hind legs. The entire upper part of the body remained normally reactive for two to three hours in the 5 frogs whose hearts had been removed, and for 1-2 days in those whose legs were ligated. Ten controls showed the usual reaction of profound depression within the expected time of 30 to 60 minutes. Later, 5 frogs with ligatures were shocked and after 30 minutes the ligatures were removed and profound depression and coma followed in a manner similar to that previously described. Later, 3 frogs were given a lethal dose of electrical injury to the hind legs. The ligatures were alternately removed and reapplied. The observations are recorded in table 1.

*Changes of the coagulation time of the blood.* Experiments to determine the effects

on blood coagulability were done by giving the animals a lethal dose of electrical injury and taking the clotting time at various intervals afterwards.

Ten animals were shocked. In  $2\frac{1}{2}$  hours 5 of the animals were opened. In 2 of the animals the clotting time was indefinite; in the remaining 3 the values were 12, 30 and 30 minutes (av. 12 minutes). After 18 hours the remaining 5 showed a range

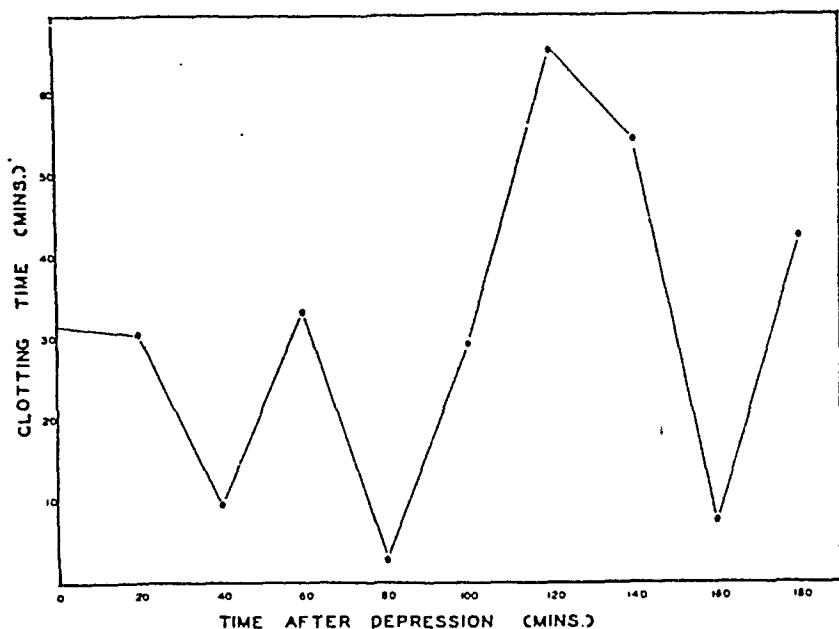
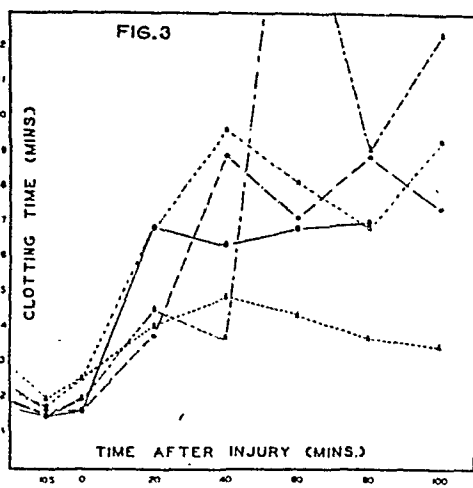
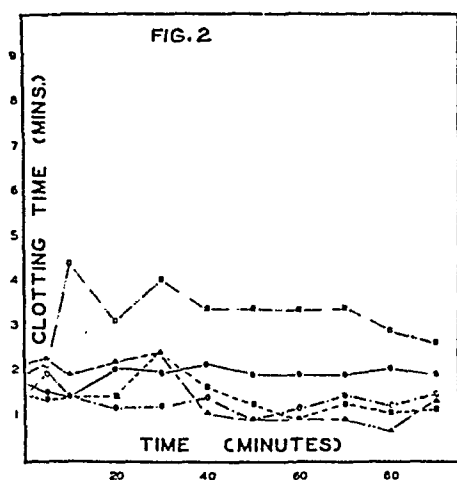


Fig. 1. EFFECT OF SHOCK due to electrical injury on the blood clotting time of frogs. Each point represents the average value obtained from the clotting time of 5 frogs at intervals after the onset of visible depression.



Figs. 2 and 3. COMPARISON OF BLOOD-CLOTTING CHANGES of control frogs (fig. 2) with those of frogs after receiving electrical injury of muscle (fig. 3). Each curve represents the changes in the clotting time of one frog. In fig. 3, samples were taken before shocking, after the 10th shock (10S), the 20th shock (o) and every 20 minutes.

of 4 to 17 minutes (av. 8 minutes). Fifteen untreated frogs, used as controls, showed a range of 1.5 to 4.5 minutes (av. 2.4 minutes).

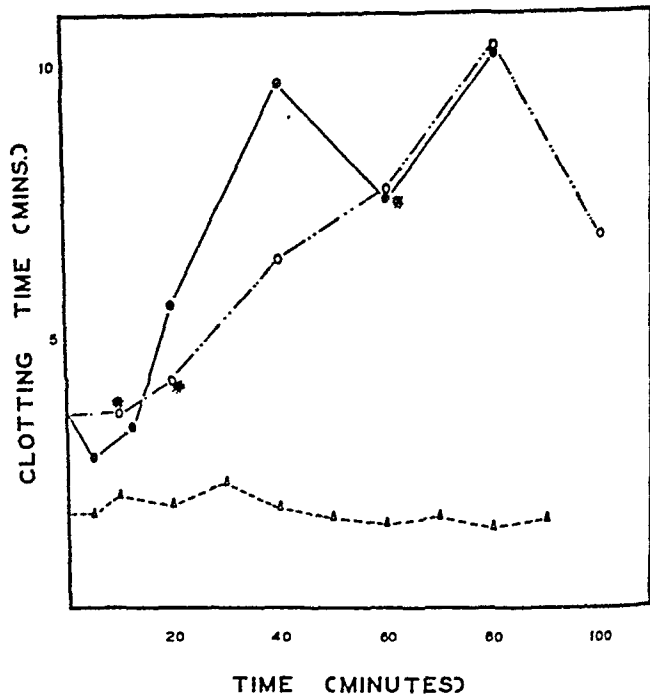
A more complete picture was obtained by shocking 50 frogs and averaging the clotting time in groups of 5 frogs at each of the following intervals: at the beginning of coma; then at 20-minute intervals thereafter for three hours. Ten controls were

used, and their average clotting time was three minutes. Results of the experiments are shown in figure 1. Indefinite clotting times were given an arbitrary value of two hours, for the purpose of averaging.

Later, serial determinations were made on 10 frogs. The results were shown in figures 2 and 4. Five untreated frogs were used as controls. The results are shown in figures 3 and 4. Figures 2 and 3 should be compared, as they show the changes of the clotting time of each animal and indicate the extent of individual variations.

In the brain injury experiments (fig. 4), 10 animals were used. The effects of electrical injury on the clotting time were substantially the same as in the muscle experiments.

Fig. 4. EFFECT OF ELECTRICAL INJURY on the clotting time of frogs. Comparison of averages obtained from test animals and controls. The asterisks (\*) represent points where the clotting time became indefinite. The curves represent only the finite values. KEY: Controls,  $\Delta$  - - -  $\Delta$ ; muscle injury,  $\circ$ — $\circ$ ;  $\bullet$ — $\bullet$ .



#### DISCUSSION

The results which have been stated give an affirmative answer to the three questions which were put at the beginning of our experiments: *Profound systemic physiological changes can be brought about in an animal by means of electrical injury to one part of the body.* This statement is in agreement with previous work done in this laboratory on other types of injury. The literature on electropathology is prodigious, but it deals chiefly with pathology caused by industrial accidents, criminal electrocution and accidental electrocutions from house currents, all of which involve excessive voltage and amperage and, often, long periods of contact with the production of high temperatures within the tissues. Another trend of recent years in the study of electropathology has been in the study of pathology resulting from electroshock therapy of psychotic patients. In this work neither the milliamperage nor the total time of application is comparable with that used in the present experiments.

However, there has been some work done to study the lethal effect of electrical injury under controlled conditions. MacMahon (5) has shown that repeated sub-

lethal doses of electrical injury can cause the death of an animal. Morrison, Weeks and Cobb (6) have studied the histo-pathological effects of various types of electrical current on the nervous system. Delayed lethal effects were produced by several applications of sublethal injury over a period of a few days. The experiments of MacMahon and Morrison and his associates were done with injury limited almost entirely to the nervous system—although MacMahon does describe some changes in skeletal muscle.

While systemic toxic effects seem to have been produced in the experiments of these workers, we felt that it was desirable to place the electrodes so that the current would not transfix the entire body, but only a relatively small portion of it.

The results of our experiments favor the second postulate, that *the toxic effects observed were caused by a toxic factor circulating within the body of the test animal*. This is in accord with results of other work done in this laboratory (4) in which the dialysate of defibrinated blood from animals in heat shock was shown to have a high toxicity for rats. Extracts of injured tissues also were found to have a lethal effect on rats. Although some normal tissue extracts are toxic, it is interesting to note that the extracts from injured tissue have a higher potency than those of normal tissue. Moreover, an indication that the toxic factor of the tissues was at least similar to, and perhaps identical with, that of the dialysate of blood is found in the fact that animals injected with these materials died in a similar way.

Our own experiments with ligatures and excision of the heart corroborate the findings of numerous investigators.

That *the circulating toxic factor is a substance with thromboplastic properties* is strongly suggested by the recorded changes in the blood-clotting time following injury. It has long been recognized that disturbances in blood coagulation follow the inception of shock from trauma, burns and anaphylactic reactions. The disturbances observed and recorded, heretofore, usually have been concerned with decrease coagulability of the blood, manifested through prolongation of the clotting time (7, 8). Often the changes in blood coagulability have been referred to as being compensatory to some postulated thrombogenic tendency incited by the pathological process involved. Actually an early decrease in clotting time has been reported, at least once, in the literature, by Gahringer (9). Thus, our experiments corroborate the findings already made by others, including the important detail of the initial increase in blood coagulability. The recent work of Dragstedt and his associates (10) showed that, following burns, there was intravascular agglutination of red cells and transient and permanent thromboses of small vessels not in the burned area. More work, on traumatic injury, has shown similar results (11). These observations, we feel, favor the view that the toxic factor is a substance which has thromboplastic properties.

The foregoing statements should not be taken to mean that the end process in shock is exclusively a matter of protoplasmic clotting. Actually, no spasticity was observed in the frogs during the development of shock. What the evidence does indicate, however, is the fact that thromboplastic changes play some rôle in the development of injury shock. Although a thromboplastic toxic factor is present, the pathological process in the target tissues might not involve overt clotting alone.

The pathology might be caused by some other reaction related to the presence of the thromboplastic toxic factor, or to the compensatory 'antithrombic' factors.

#### SUMMARY

Profound physiological depression and death were produced in the frog by means of suitable doses of electrical injury applied either to the brain or hind legs. Evidence of a circulating toxic factor was seen in the fact that the toxic effects were prevented by interruption of the blood circulation. Disturbances in the blood-clotting mechanism following injury indicate that the toxic factor is a substance, or a group of substances, with thromboplastic properties. These results favor the concept that thromboplastic substances may be involved in the pathogenesis of shock.

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# ACUTE HYPERTENSION IN DOGS WITH CEREBRAL ISCHEMIA

ARTHUR C. GUYTON<sup>1</sup>

*From the Department of Physiology, University of Tennessee School of Medicine*

MEMPHIS, TENNESSEE

*and the Department of Pharmacology, School of Medicine, University of Mississippi*

UNIVERSITY, MISSISSIPPI

CUSHING (1) demonstrated in 1903 that increased intracranial pressure can cause hypertension in dogs. Many years later Nash (2) and Volhard (3) independently showed in heart-lung-brain preparations that there is definitely a vasopressor reflex originating within the cerebral vault, and McDowall (4) demonstrated that complete cerebral anemia causes a powerful pressor response. Yet it has been the tendency by many to believe that the body's pressoreceptors are located strictly in peripheral areas (5). Experiments with sympathectomy in hypertensive patients indicate that these patients have a hyper-reactive nervous system, and there are many reasons for believing that essential hypertension is possibly neurogenic in origin (6). For this reason the present study was undertaken to elucidate the nature of the central vasopressor reflex.

## METHODS

The purpose of the surgical procedures in these studies was to abolish all reflexes originating in the carotid and aortic sinuses and thereafter to study the central vasomotor reflex resulting from ischemia of the brain. This was accomplished by two methods. In the first set of animals, all of the blood supply to the brain except that through the carotid arteries was occluded. Through an incision on each side of the neck immediately above the first rib the subclavian artery was tied at its junction with the axillary artery, and all branches from the subclavian artery were independently tied and cut. The costocervical, thyrocervical, internal mammary and vertebral arteries were occluded by this procedure. The carotid sinuses were stripped without obstructing the blood flow through the internal and external carotids, and the vagi were cut at a point approximately one inch below the carotid sinuses to denervate the aortic arch. Central vasopressor reflexes were then studied by compression of the common carotid arteries. In the second series of animals the entire bifurcation of each carotid artery, including the carotid sinus and the internal carotid up to the point of its entrance into the skull, was actually removed, and the common carotids, internal carotids and external carotids were ligated. The subclavian arteries were then isolated and tied in the neck as described for the first series of animals, and all branches of the subclavian were tied except the vertebrals. There-

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Received for publication May 10, 1948.

<sup>1</sup> Present address: University, Mississippi.

fore, the remaining blood supply to the brain flowed entirely through the vertebral arteries except for collateral supply through spinal and muscular channels. The cerebral reflexes were then studied by compression of these vertebral arteries. A special screw clamp was devised for this purpose so that the incisions could be closed except for the projection of a small brass tube and screwing mechanism.

Because the respiration of the animals often ceased when the brain became ischemic, it was necessary to insert an intratracheal cannula for artificial respiration. The animals were anesthetized with sodium pentobarbital to a stage of medium surgical anesthesia. The experiments progressed over a period of 4 to 7 hours. During this time, approximately one half of the animals suffered a reduction in blood pressure below normal because of the extensive operative procedures. Nevertheless, these animals had essentially the same responses found in the other animals though quantitatively smaller. To insure that the carotid sinus areas were completely denervated, they were painted after stripping with a 1 per cent solution of phenol and electrically stimulated to prove that no further neurogenic connections existed from these areas. Blood pressure was recorded from a femoral cannula.

#### RESULTS

After standardizing the surgical procedures, various studies of the vasopressor reflexes were made in 16 animals as follows:

A. *Acute occlusion of the common carotids with the remaining cerebral circulation intact* produced the well-known results which are generally ascribed to the carotid sinus reflex. Clamping of either one of the common carotids produced a rise in blood pressure of approximately 6 to 10 mm. Hg. Occlusion of both carotids simultaneously produced an average rise in 12 animals of 26 mm. Hg. This rise in blood pressure was maintained for the duration of carotid occlusion regardless of how long the carotids were compressed, and the blood pressure returned to normal immediately after release of the clamps.

B. *Acute occlusion of the common carotids after the vertebrals and other branches of the subclavian had been tied* produced, in 8 animals, an average rise of blood pressure of 57 mm. Hg. The character of this rise in blood pressure was slightly different from that obtained before the vertebrals and accessory circulation had been occluded. In general, the blood pressure rose rapidly for 15 seconds, then slowly for approximately one minute, after which time there was a slow fall over a period of 8 to 15 minutes back to normal or below normal. At the end of each period of occlusion there was usually a 30-second period of compensatory subnormal pressure ranging 20 to 30 mm. below normal. Cerebral ischemia in these experiments was complete except for minor blood flow through collateral channels. For this reason, even the vasopressor centers probably became functionally inactivated after a few minutes, resulting in the slowly falling pressure. The greater rise in blood pressure after the vertebrals had been ligated than before could be explained by two possible mechanisms. First, there is the possibility that back pressure in the carotid sinuses, from the circle of Willis, was less in the second case. Second, there is possibly a pressoresponsive area located in the brain in addition to those in the peripheral circulation.

C. *Acute occlusion of the common carotids with the vertebrals tied and carotid*

*sinuses denervated* still caused a rise of blood pressure averaging 30 mm. Hg in 7 animals. In this instance the carotid sinuses were completely inoperative and the aortic sinuses, though still intact, were actually opposing the rise in blood pressure. The character of the blood pressure response was essentially the same as that noted before the carotid sinuses were denervated, though the height was less and the rapidity of rise was slightly decreased. Likewise when the clamps on the carotids were removed, the blood pressure fell rapidly and markedly to a level 20 to 30 mm. below normal, returning thereafter to normal in approximately 15 to 30 seconds.

D. *Acute occlusion of the common carotids with the vertebrals tied, the carotid sinuses denervated and both vagi cut* caused a rise in blood pressure averaging 46 mm. of mercury in 6 animals as shown in table 1. The blood pressure, after the vagi were cut, rose much more rapidly than before, reaching a maximum in approximately 30 seconds rather than in one minute and 15 seconds. The height of the pressure was approximately one and one-half times the blood pressure rise before the vagi were cut. Cutting the vagi removed two factors in the neurogenic control of blood pressure. First, it removed the tonic and reflex effect of the vagi on the heart. This caused a variable rise in blood pressure at the time of cutting the vagi. Second, cutting the vagi denervated the aortic arch. This removed the buffering action of the pressoreceptors of the aortic arch and resulted in a higher and more rapid rise in pressure after carotid occlusion. Blood pressure readings in this category were often over 200 and, in one animal, reached a mean pressure of 260 mm.

E. *Acute occlusion of the vertebrals with the carotid sinuses totally ablated and the carotids, subclavians, costocervicals, and thyrocervicals all tied* produced an average rise in blood pressure in 4 animals of 15 mm. Hg. In this instance the carotid sinuses had been completely removed and, therefore, could have had no effect whatsoever in producing this rise in blood pressure. The character of the rise in blood pressure was the same as that previously described when the brain was almost completely ischemic except that the total rise was less than that previously discussed.

F. *Acute occlusion of the vertebrals in the 4 animals noted in paragraph E after both vagi had been cut* caused a rise in blood pressure averaging 32 mm. Hg. In this set of observations, in which both the action of the vagi on the heart and the buffering action of the aortic arch had been removed, the result was an exaggeration of the central vasopressor response.

G. *The entire neck except the spinal column was sectioned in two of the animals which already had both carotid sinuses ablated, the carotids, subclavians, costocervicals and thyrocervicals all tied, and the vagi cut.* After this procedure, the average rise in blood pressure on occlusion of the vertebrals was 76 mm. Hg as noted in table 1. It appeared that the collateral circulation increased much more rapidly when the experiment was carried out by ablating the carotid sinuses first rather than by tying the vertebrals first. This is reasonable because the small vertebrals could not adequately supply the entire head with blood. While sectioning the neck, even though the carotids had been tied and the vertebrals clamped, numerous small but profusely bleeding arteries were found throughout the muscles.

H. *Control observations.* After the buffering action of the carotid sinuses and the aortic arch had been removed, it would be possible for the increase in peripheral



resistance upon clamping the carotids to be partly responsible for the rise in blood pressure. Therefore, in 4 of the animals which had had the carotid and aortic sinuses denervated, one femoral artery was suddenly occluded. In not one of these 4

TABLE 1. EFFECT OF OCCLUDING THE CEREBRAL BLOOD SUPPLY AFTER THE CAROTID SINUSES AND THE AORTIC ARCH HAD BEEN DENERVATED

DOG NO.	VESSELS OCCLUDED	DURATION OF OCCLUSION	B. P. BEFORE OCCLUSION	MAXIMUM LEVEL OF B. P. DURING PERIOD OF CEREBRAL ISCHEMIA	REMARKS
		<i>min.</i>		<i>mm. Hg</i>	
5	carotids	13	140	166	Pressure had fallen to 105 at 13 min.
6	carotids	4	156	206	Pressure fell to 110 on release of occluding clamps
		14	150	226	Pressure had fallen to 70 at 14 min.
7	carotids	3	154	184	Fell to 130 on release of clamps
		10	160	214	Pressure maintained, respiration did not cease
8	carotids	5	104	140	Rapid fall to 70 after release
9	carotids	2	166	234	Rapid fall to 130 after release
		3½	154	224	Same rapid fall
		25	184	260	Sustained blood pressure rise and sustained respiration
10	carotids	2	174	234	Rapid fall to 130 after release
		4	154	220	Same rapid fall
		22	170	200	After tying external carotids. Sustained rise in blood pressure and sustained respiration
14	vertebrals	3	100	150	Slow fall back to normal after release of clamps
		7	94	172	Slow fall back to normal after release of clamps
15	vertebrals	1½	60	168	Slow fall after release of clamps
		2	104	170	Slow fall after release of clamps

In animals 5, 6, 7, 8, 9, and 10, the vertebrals, costocervical, thyrocervical and subclavian arteries had been ligated. In animals 14 and 15, the carotid bifurcations had been removed and the necks had been entirely sectioned except the vertebral arteries and the spinal column. The blood pressure levels reached their maximum height between 30 sec. and 5 min. after which they usually began to fall at a very slow rate.

animals was there a perceptible rise in blood pressure although the blood flow through the femoral was approximately equal to one half the total blood flow through both carotids and equal to considerably more than the blood flow through both vertebrals.

Tying the external carotids in 3 animals did not qualitatively change the cerebral

vasopressor response on occlusion of the carotids. Quantitatively, however, the response was slightly decreased. Because the internal carotids in dogs are extremely small arteries in comparison with the external carotids, it is reasoned that the brain probably receives a considerable proportion of its blood supply through anastomoses from the external carotids as well as through the internal carotids.

At the conclusion of one of the experiments in which the animal had had its entire neck sectioned and in which the carotid arteries as well as the vertebral arteries had been ligated, the entire spinal column was rapidly sectioned between vertebrae C 2 and 3. Although both vertebrals had been ligated at their origin immediately above the first rib, the animal bled to death through these vertebrals in approximately two minutes, thus showing that there was still a marked anastomotic supply between the cephalad end of the vertebrals and blood vessels of the thoracic region. This could easily have occurred because the neck had been sectioned in the region of C 2 and 3 rather than at the point at which the vertebrals had been tied. The collateral blood supply to the brain appears to be exceptionally well developed. This makes it difficult to state the precise degree of cerebral ischemia which occurs after carotid and vertebral ligation.

I. *The effect of cerebral ischemia on respiration* varied with the duration and degree of ischemia. In approximately two thirds of the animals having the carotids, the vertebrals and accessory blood vessels occluded, respiration ceased between 45 seconds and 8 minutes after occlusion. In the other one third of the animals the respiration continued indefinitely at a slow rate. The degree of hypertension produced by cerebral ischemia was greatest in those few animals which were on the verge of total respiratory arrest but did not actually stop breathing. Likewise, the pressure response in these animals was sustained over a longer period of time than in the others. This indicates that there is a particular point in cerebral ischemia at which high levels of blood pressure can be maintained, whereas greater ischemia than this will cause a fall in blood pressure due to functional inactivation of those cells which cause the vasopressor response. During all periods of respiratory arrest, artificial respiration was instituted to prevent systemic anoxia.

J. *Prolonged cerebral ischemia in those animals which experienced complete respiratory arrest* caused, within a period of 8 to 14 minutes, a complete medullary paralysis. The blood pressure levels by this time had fallen to approximately 70 mm. Hg and clamping or releasing the arteries to the brain caused no further blood pressure responses. The animal was thereafter essentially a spinal animal and of no further use for these acute experiments.

K. *The pulse rate* in total cerebral ischemia invariably decreased after approximately 45 seconds of arterial occlusion. Before the vagi were cut, this decrease was often as much as 30 per cent. It was still present, however, even after the vagi were cut, though usually around 5 per cent in these instances rather than up to 30 per cent. Before the vagi were cut there also was often a rapid rise in blood pressure for the first 30 seconds, a small fall in blood pressure for the next 30 seconds, and then a secondary rise to higher levels at  $1\frac{1}{2}$  minutes. After cutting the vagi this blood pressure dip was still noted to a slight degree in several of the animals, and it was associated with mild slowing of the pulse rate.

## DISCUSSION

Long before the description of the carotid sinus reflex, the pressor response elicited by cerebral ischemia had become well known and was extensively reviewed by Winkin (7). Most of these experiments became invalidated with the discovery of the carotid sinuses, because clamping of the common carotids, which was almost always the experimental technic, caused both the cerebral ischemic response and the carotid sinus reflex response. McDowall, however, in a series of experiments designed to study the chemical control of the vasomotor center, demonstrated that a striking elevation of blood pressure could still be obtained by occluding the blood supply to the brain peripheral to the carotid sinuses and that the response persisted after the carotid sinuses had been denervated (4). It has been the purpose of the present set of experiments to evaluate this cerebral ischemic pressor response in relation to the better known peripheral pressoreceptor reflexes.

Comparison of the results of cerebral ischemia produced by arterial occlusion with those of ischemia produced by increased cerebrospinal fluid pressure in Cushing's experiments (1) is striking. There is the same slow rise in blood pressure in both procedures as well as the vagal slowing of pulse rate in the early stages. Likewise, there is the same blood pressure dip which often occurs simultaneously with the pulse slowing. The changes which take place after cutting the vagi are also the same, that is, the blood pressure rise is much more rapid, and the vagal slowing and early blood pressure dip are almost completely abolished. Cushing's original conclusion, that increased cerebrospinal fluid pressure causes the blood pressure response by producing cerebral ischemia rather than by some other mechanism, agrees quite accurately with observations in the present experiment.

Location of the centers responsible for the pressor response in the cerebral vault might be in the actual cerebral nuclei or possibly in the arterial system of the brain. It is well known that stimulation of certain areas of the hypothalamus, the mesencephalon, the pons and the medulla will cause either a rise or fall in blood pressure depending on the point of stimulation (8). It is therefore reasonable to assume that the location of the vasopressor centers concerned in the present observations could be in one of these areas. The fact that respiratory depression and rise in blood pressure correlate very closely indicates that the medulla is the area possibly concerned.

The presence of *arterial* pressoreceptor areas, similar to the carotid sinuses, inside the cerebral vault is untenable for two reasons. First, the rise in blood pressure in cerebral ischemia is usually somewhat slower than the rapid carotid sinus response, and the ischemic response often is not maintained as is the carotid sinus response. Second, characteristics of the blood pressure response in cerebral ischemia are exactly the same as those which occur when the cerebrospinal fluid pressure is increased. In this latter instance the applied pressure is external to the blood vessels, and the blood pressure should fall rather than rise if pressoreceptor areas similar to the carotid sinuses should exist within the arterial tree of the brain.

Whether the pressor response is due to actual pressoreceptor nuclei or to chemoreceptor nuclei responding to metabolic effects of hypotension is impossible to state.

The long, slow rise in blood pressure seen in the responses of figure 2 would imply a chemoreceptor system. Also, McDowall's work (4) on the chemical control of the vasomotor center indicates that carbon dioxide concentration is an important factor. On the other hand, very rapid responses occurred in a few animals as illustrated in figure 1d. These responses are more characteristic of a pressoreceptor system but not necessarily so. It is difficult to imagine the mechanical construction of a nerve

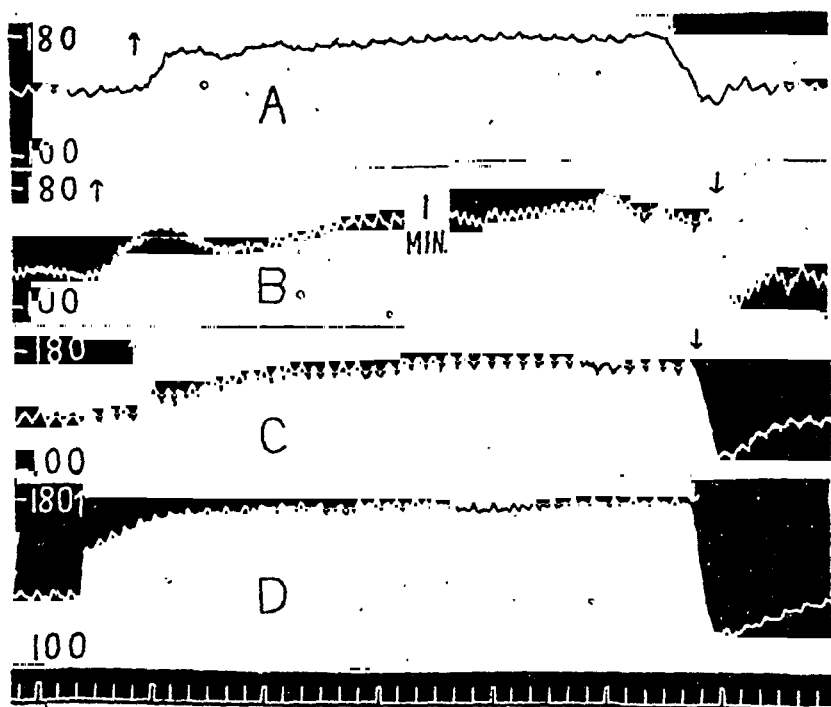


Fig. 1. BLOOD PRESSURE RESPONSES during progressive stages of the experiment: *a*) Clamping of both common carotids with the animal otherwise intact; *b*) clamping of both common carotids with the vertebrals tied; *c*) clamping of both common carotids with the vertebrals tied and the carotid sinuses denervated; *d*) clamping of both common carotids with the vertebrals tied, the carotid sinuses denervated and the vagi cut. Note in *b* the dip in blood pressure at approximately 40 seconds; this occurred frequently when the brain was almost totally deprived of blood flow but was usually entirely abolished after the vagi were cut. Note, also, the compensatory subnormal pressures after the clamps were removed from the carotids. (Time intervals—5 sec.)

cell which can respond to pressure, though such is not an impossibility. Regardless of which type of system is responsible for the pressor response, the activity of the system is rapid enough to be of protective value in animals with a falling blood pressure.

It is probable that the pressor response in cerebral ischemia is mediated through the sympathetic nervous system. The very rapid and marked fall in blood pressure when the clamps are removed from the occluded arteries indicates that the response is neurogenic rather than humoral. The vagi, of course, had been cut in these experiments and therefore could not have been concerned. Also, it has been adequately demonstrated that the rise in blood pressure due to the similar condition of increased cerebrospinal fluid pressure is mediated through the sympathetics (1, 9). Further-

more, McDowall (4) demonstrated by several methods of cerebral asphyxia that the response is opposed by removal of portions of the sympathetics.

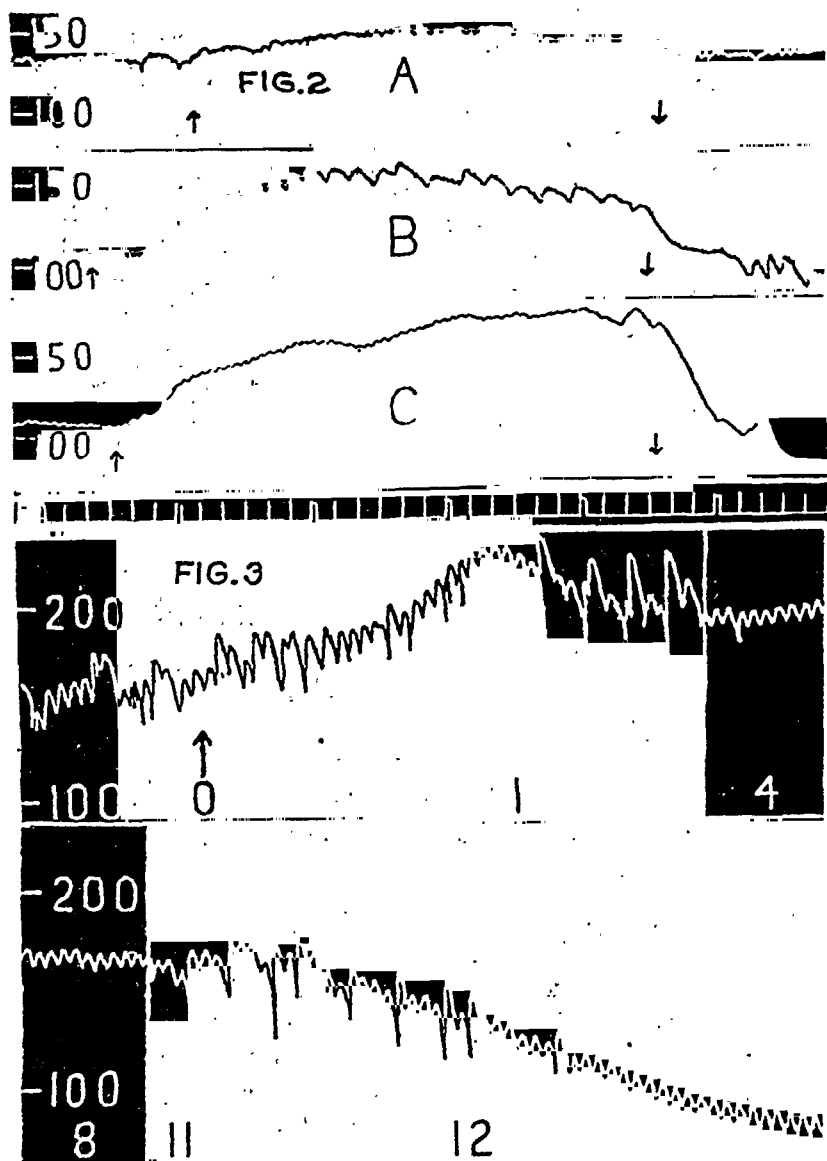


Fig. 2. BLOOD PRESSURE RESPONSES after the carotid sinuses had been totally ablated: a) Clamping of both vertebrals with both carotid bifurcations including the sinuses removed; b) clamping of both vertebrals with both carotid sinuses ablated, the vagi cut and part of the soft tissues of the neck sectioned; c) clamping of both vertebrals with both carotid sinuses ablated, the vagi cut and the neck, except the spinal column, completely sectioned. Note the change in response as the collateral circulation was destroyed and the aortic sinus denervated. (Time intervals—5 sec.)

Fig. 3. BLOOD PRESSURE EFFECT of prolonged cerebral ischemia. Medullary paralysis with resultant spinal levels of blood pressure occurred in 8 to 14 minutes in all animals if the ischemia was sufficient to cause respiratory arrest.

The present experiments indicate forcefully that the peripheral pressoreceptors are not the only major neurogenic pressoresponsive system. Indeed, it is even questionable whether the carotid and aortic sinuses are as powerful as the cerebral centers.

It is regrettable that many of the experiments to produce chronic hypertension by carotid sinus denervation have been carried out by actual ablation of the carotid bifurcation (10-12). This procedure could easily cause a low-grade deficiency in blood supply to the brain, and what the effects of this may be have not been determined. For this reason, and because the results of chronic experiments with sinus denervation have been extremely variable (10-14), the precise function of the carotid sinuses, the aortic arch and the mesenteric pressoreceptors (15, 16) is still quite beclouded.

The relation of these experiments to essential hypertension in man is of interest. Certainly, no one has succeeded in implicating the carotid sinus mechanism as a causative factor. Yet, excessive neurogenic pressor responses in such patients have been demonstrated by postural and ice water tests (6). Also, it has been shown many times that spinal (17), caudal (18), and differential block (19) anesthesia, which effectively block the sympathetic nervous system, will cause a marked blood pressure fall in many hypertensive patients, whereas these procedures hardly affect the blood pressure of normal individuals. These observations indicate a cerebral origin of excess sympathetic activity in hypertensive patients. Because stimulation of an extremely small area of the medulla has been shown to cause extreme changes in blood pressure (8), there is no reason to doubt that local vasospasm or arteriosclerotic occlusion of a small blood vessel might be responsible for the hyperactive sympathetics. Indeed there might well be a vicious cycle with localized vasospasm initiating a neurogenic hypertension and this in turn reflexly increasing the vasospasm.

Recent war experiments concerning the effect of increased gravitational force on the body indicate that man's ability to rapidly adjust the blood pressure under changing forces is more highly developed than that of lower animals (20). This is reasonable because of man's erect posture, and it might also explain why essential hypertension is principally a disease of mankind.

Chronic hypertension has been produced in dogs by progressively occluding the arterial blood supply to the brain (21). To cause this state, it has been necessary to occlude even the anterior spinal artery, and it might be reasoned that such extreme ischemia could hardly exist under natural conditions. One must remember, however, that very minute areas of the brain may exert powerful pressor effects, and severe localized ischemia in these areas can easily occur.

#### SUMMARY

A marked rise in blood pressure occurs in response to acute cerebral ischemia. This response is still present after reflexes from the carotid sinuses have been abolished. Quantitative data indicate the cerebral pressoreceptor response to be as powerful as the carotid sinus response though of a slightly different character. Respiration is also depressed by acute cerebral ischemia, and the rise in blood pressure generally is greatest when the respiration is barely present. Prolonged cerebral ischemia produces complete medullary paralysis causing the blood pressure to fall to levels of a spinal animal. It is postulated that the centers responsible for the blood pressure response are located in the medulla. The blood pressure response due to cerebral ischemia is almost identical with that shown by Cushing to occur

in increased cerebrospinal fluid pressure. The possible relationship of these observations to essential hypertension is discussed.

I wish to express my appreciation to Dr. J. P. Quigley for his helpful suggestions in this project and to Mrs. Gene Hawkins, Mr. D. H. Cates, Mr. W. B. Beasley and Mr. J. G. Hunt for their technical help in the operative and recording procedures.

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# ELECTRICAL STIMULATION OF THE NEURAL MECHANISM REGULATING SPASMODIC RESPIRATORY ACTS IN THE CAT<sup>1,2</sup>

HERBERT LEON BORISON

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University*

NEW YORK CITY

IN 1939 Wang and Ranson (1) mapped the reactive points for certain autonomic responses to stimulation of the cat's brain stem and reported as an incidental observation an unusual augmented respiratory response elicited at the level of the calamus scriptorius. Since then Pitts, Magoun, and Ranson (2), Brookhart (3) and others (4, 5) have carefully explored the medulla for various respiratory effects but have made no mention of this particular type of response.

During the past year, the medulla oblongata of the cat has been stimulated in this laboratory with the aid of the Horsley-Clarke stereotaxic instrument, and a spasmodic respiratory response similar to that reported previously by Wang and Ranson (1) has been obtained. This recent work indicates that the response may be further characterized as a rhythmic explosive expiratory act as typically seen in coughing, sneezing and retching. It has been repeatedly and consistently elicited on stimulation of definite loci in the dorsolateral portion of the medulla. The purpose of this work is to localize the neural structures involved, to study in detail the physiological nature of the response, and to determine the conditions favoring its occurrence.

## METHODS

Of the 40 experiments performed in this study, 25 were done on cats anesthetized with nembutal (20 mgm. per kgm.) given intraperitoneally. This was usually supplemented with ether during the operative procedure. The remaining 15 experiments were carried out on decerebrate cats prepared by midcollicular transection (6) under ether anesthesia. The cerebellum was exposed widely by removing with trephine and rongeurs the portion of the occipital bone from the foramen magnum to the lambdoidal ridge. The interior of the medulla was stimulated with a bipolar enamelled wire electrode which was oriented by means of the Horsley-Clarke stereotaxic instrument (7). The electrode was mounted in a rotatory electrode carrier (8) and was inserted through the cerebellum into the medulla at a forward inclination of 15 degrees. The stimulating current was supplied by a thyatron stimulator (9) which was calibrated with a cathode ray oscilloscope. Since, as will be shown, it was necessary to leave the glottis undisturbed, a pneumograph type of recording offered certain advantages over the use of a spirometer. Taking into consideration the misleading effects which extraneous body movements frequently produce in ordinary pneumograph tracings, as was pointed out by Pitts *et al.* (2), a system was devised in which blood pressure cuffs (8 cm.) were adapted as thoracic and abdominal pneumographs; these were used in conjunction with gravity writing tambours. That this method gave reliable recordings is indicated in figure 1, in which simultaneous pneumograph and spirometer records made under a variety of conditions are shown. At the termi-

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Received for publication May 24, 1948.

<sup>1</sup> A preliminary report of this work appeared in *Federation Proc.* 7: 10, 1948.

<sup>2</sup> Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.



nation of each experiment the cat's brain was perfused first with saline, then with formalin to fix the tissue in situ. This technique reduced to a minimum any distortion of the brain which might occur on subsequent handling. The points reactive to stimulation in each medulla were localized by identifying the electrode punctures in Weil-stained serial sections cut in the plane of the punctures.

### RESULTS

In addition to the maintained inspiratory and expiratory responses localized by Pitts and his co-workers, faradic stimulation of the lower brain stem has yielded a characteristic spasmodic respiratory response which is specific for the dorsolateral region of the myelencephalon.

*Spasmodic respiratory response.* To appreciate the actual nature of the spasmodic response it was necessary to avoid the use of a tracheal cannula so that the glottis could function normally. With the glottis intact, the response may be readily distinguished as a cough, sneeze or retch. Typical examples of the recorded spasmodic respiratory response are presented in figures 2 and 3. The response occurs only during the stimulation period although it may terminate before the end of stimulation. Those responses which appeared after the stimulation time were not considered typical and were not included in the localization of the reactive points. The spasmodic response consists of strong inspiratory and expiratory efforts usually occurring at a rate of one per second, but in some instances taking place at rates even as slow as one every four seconds. While the glottis is a useful indicator of the specific character of the spasmodic respiratory act, bypassing it with a tracheal cannula does not alter the recorded response to any appreciable extent. Similar responses to those elicited on direct stimulation of the medulla may be obtained by stimulating certain afferent nerves. In figure 4 the responses resulting from stimulation of the central ends of the glossopharyngeal and superior laryngeal nerves are presented for comparison with those obtained by medullary stimulation.

*Localization.* The results of histological identification of the reactive points from which the spasmodic respiratory response was obtained are compiled in the drawings of figure 5. These represent sections of the medulla spaced at approximately 1.5 mm. intervals extending from the level of the 6th cranial nerve caudad to the level of the 12th cranial nerve. In order to make the localization as exact as possible, only those points which gave maximal responses are plotted. It is seen that the responsive areas correspond to the descending vestibular tract and nucleus, the tractus solitarius and its nucleus, and the entering glossopharyngeal and vagal rootlets. Only sporadic or questionable responses were obtained either rostral or caudal to the levels indicated in figure 5. The levels at which the reactive points are most concentrated are *B*, *C*, and *D*, corresponding to the outflows of the 9th and 10th cranial nerves. Although the same total number of points were not stimulated at each level, the data of figure 5 give a rough idea of the relative reactive density within the responsive region localized. It is not possible with the present information to subdivide the responsive region into distinct parts each separately responsible for coughing, sneezing and retching. Changes in position of the electrode in the immediate vicinity of the localized region gave no predictable pattern of responses. Thus, there were times when on moving the electrode dorsad through the medulla, the spasmodic respiratory response would appear following a typical apneusis, and on

Fig. 1. COMPARISON OF SPIROMETER and pneumograph tracings. *Top tracing*: spirometer, inspiration downstroke; *center and bottom tracings*: thoracic and abdominal pneumographs respectively, inspiration upstroke. A. Maintained inspiratory response; B. expiratory apnea; C. spasmodic response; D. atypical delayed spasmodic response. Time signal: 15 seconds.

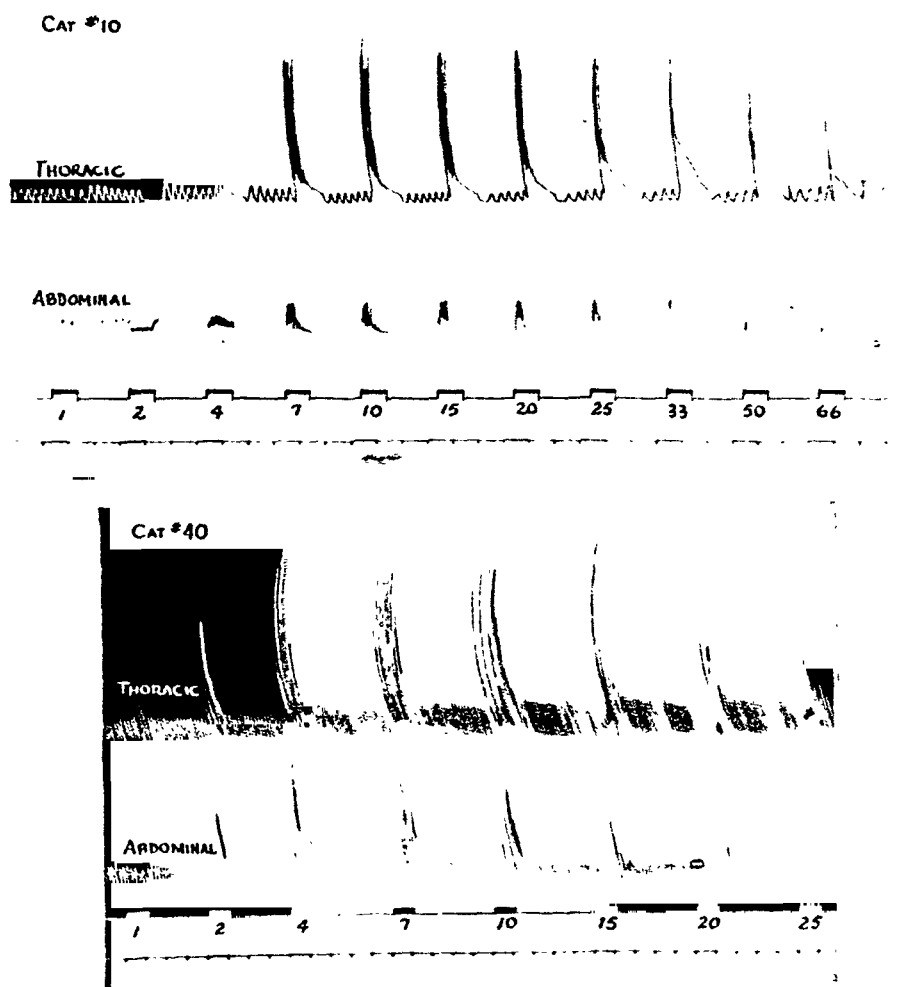
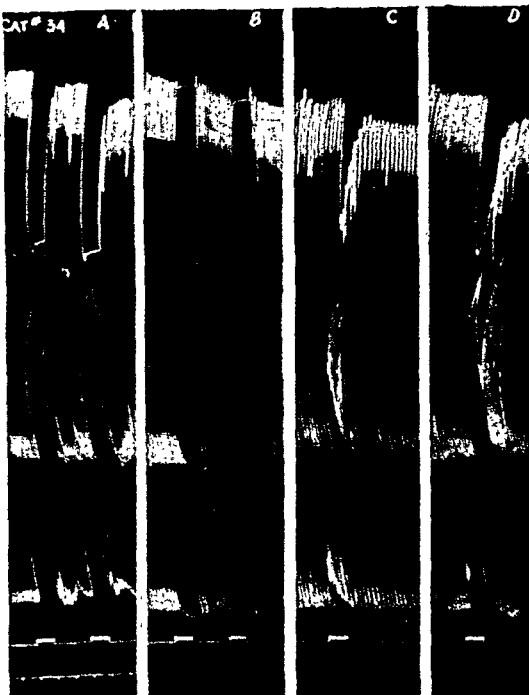


Fig. 2. EFFECT OF frequency variation of the stimulating current on the spasmodic respiratory response. Frequency, in impulses per second, indicated by the numbers under the stimulation marker. Time signal: 15 seconds. *Upper record*: cat 10, voltage is constant at 12 volts. Optimal range of frequencies is from 7 to 25 impulses per second. Note at high frequencies the inspiratory cramp in the thoracic tracing and the expiratory apnea in the abdominal tracing. *Lower record*: cat 40, voltage is constant at 8 volts. Optimal range of frequencies is from 2 to 10 impulses per second.

other occasions it might occur immediately superficial to a point yielding an expiratory apnea. In any event, the spasmodic response was always elicited most dorsally.

*Frequency and voltage characteristics.* The spasmodic respiratory response can be elicited solely within well defined and relatively small limits of frequency and voltage variation of the stimulating current. It can be seen from figure 2, *cat 10*,

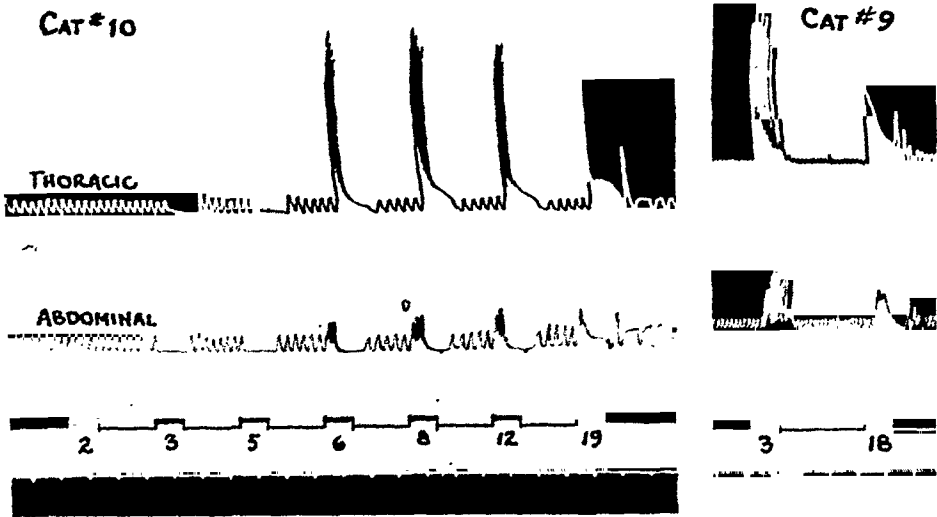


Fig. 3. EFFECT OF voltage variation of the stimulating current on the spasmodic respiratory response. Frequency is constant at 15 impulses per second in both records. Voltage is indicated in volts by the numbers under the stimulation marker. Time signal: 15 seconds.

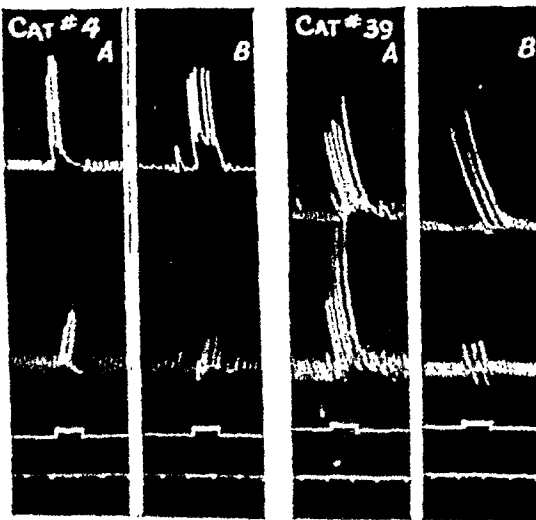


Fig. 4. COMPARISON OF SPASMODIC RESPIRATORY RESPONSES resulting from direct medullary stimulation and the stimulation of afferent nerves. *Upper tracings:* thoracic pneumograph; *lower tracings:* abdominal pneumograph. Time signal: 15 seconds. *Cat 4*, A. Medullary stimulation at 25 volts and 10 impulses per second; B. glossopharyngeal nerve stimulation at 25 volts and 10 impulses per second. *Cat 39*, A. Medullary stimulation at 8 volts and 4 impulses per second; B. superior laryngeal nerve stimulation at 18 volts and 4 impulses per second.

that with a constant current strength of 12 volts, the response is optimal in the frequency range of 7 to 25 impulses per second. At the low frequency end, the spasmodic response appears suddenly, whereas at frequencies above 25 impulses per second it is restrained and replaced by an inspiratory cramp. Variation in voltage gives results which are similar to the effect of frequency change (fig. 3, *cat 10*). With constant frequency at 15 impulses per second the response appears suddenly at 6 volts

and at 19 volts is merged into an inspiratory cramp. It should be pointed out that the results cited are for a single experiment and that there are differences in the optimal range of frequency and voltage from animal to animal (fig. 2, *cat 40* and fig. 3, *cat 9*). Nevertheless, it must be emphasized that the range of reactive frequencies is consistently below 40 impulses per second and in any given animal lies within fairly restricted limits. The effects of voltage variation are not as specific as those produced by frequency change, yet it is significant that the response is elicitable at moderate current strengths. Anesthesia is an important factor to be controlled, for the depth of anesthesia has a strong modifying effect on the current characteristics and may possibly cause complete disappearance of the response.

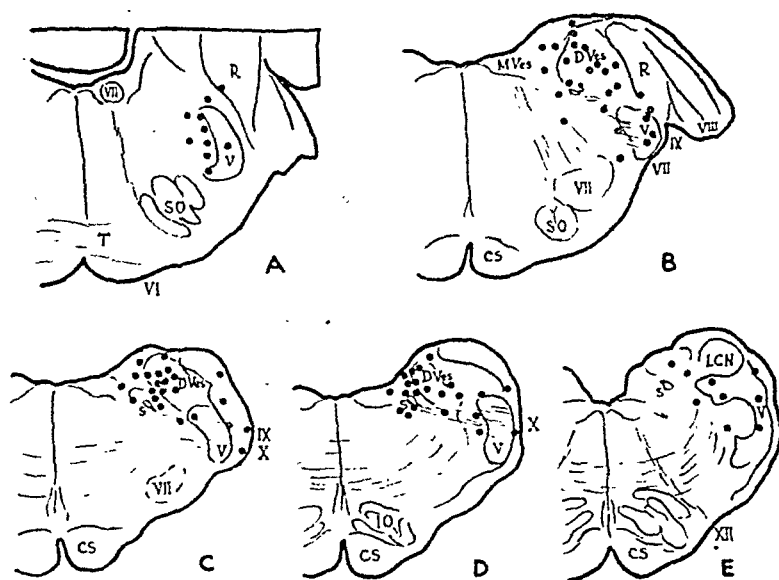


Fig. 5. SECTIONS OF THE MEDULLA of the cat spaced at approximately 1.5 mm. intervals between the levels of cranial nerves VI and XII. *Solid circles*: maximal spasmodic respiratory responses. Ratio of positive to negative experiments at each level: A, (4/7); B, (11/0); C, (6/2); D, (8/5); E, (2/3). *DVe*s, descending vestibular tract and nucleus; *MVe*s, medial vestibular nucleus; *R*, restiform body; *s*, tractus solitarius and nucleus; *SO*, superior olivary nucleus; *IO*, inferior olivary nucleus; *T*, trapezoid body; *CS*, corticospinal tract; *LCN*, lateral cuneate nucleus.

For routine exploration, a stimulating current at a frequency of 15 impulses per second and a strength of 8 volts was employed. In general, high frequency or voltage stimulation causes the spasmodic response to be converted into a characteristic inspiratory cramp, and low frequencies and voltage tend to change it into an expiratory apnea. However, numerous exceptions to this generalization have been seen. A possible effect of voltage or frequency variation which has been considered but never observed is the conversion of one type of spasmodic respiratory response to some other type, for example, coughing to sneezing.

#### DISCUSSION

Lumsden in 1923 (10) suggested the existence of a medullary center for active expiratory efforts similar to coughing and sneezing. His basis for this assumption is that these sudden forced respiratory acts are obtainable on stimulation of afferent fibers in the 5th and 10th cranial nerves, and that the neural structures above the

level of the striae acousticae are not essential for these acts. In more recent years, the brain stem has been explored extensively for respiratory reactivity by direct electrical stimulation (2-5) with the sole criterion being respiratory movement for the purpose of pulmonary ventilation. That the respiratory apparatus is also normally utilized for other important functions, as for expulsive purposes, is obvious. These later investigators have in general used stimulating currents with frequencies too high to elicit the spasmodic respiratory response observed in this study. Moreover, if the trachea is cannulated for spirometer recording, thus bypassing the glottis, it is quite difficult to recognize the nature of the spasmodic respiratory response and practically impossible to distinguish between the various types of spasmodic acts.

Although pneumograph recording gives only qualitative information it has the advantage of not interfering with respiratory function, and also provides a means of separating the thoracic and abdominal components of respiration. The rôle of the abdominal musculature is of particular importance in expulsive respiratory acts. Contrary to the claim of Pitts (2) that the pneumograph cannot be used with accuracy, this recording method has been shown to faithfully reproduce a variety of respiratory activities (fig. 1).

Using appropriate stimulus frequencies and strengths, the spasmodic respiratory center has been localized within relatively sharp boundaries in the periventricular substance of the lower brain stem. It is apparent from the illustrations in figure 5 that the distribution of the reactive points is sparser in the most caudal and rostral drawings than in the levels *B*, *C* and *D*. This indicates that levels *A* and *E* represent the outlying limits of the region which has been defined by electrical stimulation as regulating spasmodic respiratory responses. Those structures implicated in the localization correspond mainly to the descending vestibular root and its nucleus, the tractus solitarius and its nucleus and related vagal cells. It has been shown by Pitts (11) that the method of localization used in this study is accurate to within half a millimeter. This means that while the method has inherent limitations, it is sufficiently exact to permit the designation of specific structures with reasonable certainty. A comparison of the localization of the spasmodic respiratory center with Pitts' respiratory center shows a striking lack of conflict between the anatomical positions of the two centers. There appears to be very little overlap at those levels in which they are coexistent.

Undoubtedly some of the responses elicited from the reactive points are the result of the stimulation of afferent fibers and tracts. Lumsden has induced expulsive responses by electrical stimulation of the 5th and 10th nerves or by blowing air into the nostril or larynx. Teitelbaum and Ries (12), who stimulated the glossopharyngeal nerve, described the response as an immediate marked respiratory augmentation. The recordings presented in figure 4 leave no question as to the close similarity between the responses obtained reflexly and those resulting from direct stimulation of the reactive region in the medulla. Nevertheless, the fact that the responsive points are concentrated within a well defined region, and that the response is still centrally elicitable after midbrain decerebration, is evidence for the existence of a spasmodic respiratory center in the rhombencephalon. This view is further strengthened by an experiment performed by Lumsden in which the forced expiratory

response was produced reflexly after decerebration, demonstrating that the central coordinating mechanism must still be intact. Furthermore, direct stimulation of the pontile region gave responses which did not satisfy the criteria for spasmodic respiratory acts, thereby serving to limit the rostral extent of the spasmodic respiratory center to the caudal edge of the pons. Having thus restricted by the process of elimination the central mechanism for spasmodic acts to the myelencephalon, one must conclude that the spasmodic respiratory center lies within the reactive region shown in figure 5.

It should be emphasized that the spasmodic response can be elicited only within very specific frequency and voltage limits of the stimulating current. Although low frequency stimulation is a necessary condition for the production of the spasmodic response, the latter is not an invariable consequence nor is it an artifact associated with such stimulation. Structures outside the spasmodic respiratory center when stimulated with a low frequency current yielded no similar results. Indeed, Pitts has previously pointed out that reducing the frequency or voltage of stimulation in the medial reticular formation does not vary the form of the maintained respiratory response but merely decreases its amplitude. This is in contradistinction to the marked altering effect which variation of frequency or voltage has on the spasmodic response. It is evident from the foregoing that the frequency and voltage characteristics necessary for eliciting the spasmodic respiratory response give it a firm basis as a physiological entity. In other words, the spasmodic response represents a real and coordinated action of the respiratory musculature specifically obtainable with a specialized stimulus from well defined medullary structures.

There can be no doubt as to the close synaptic relationship of the spasmodic respiratory center with the medullary center localized by Pitts and his associates (2). Evidently there is a neuronal mechanism within the medulla which rhythmically activates the inspiratory and expiratory cells in the production of forced spasmodic acts. At the present time the importance of this mechanism in the regulation of normal rhythmic breathing can only be conjectured. Lumsden (13) postulated a pneumotaxic center in the pons. Later investigators (14-16) have further established the importance of the pneumotaxic center as a source of periodic inhibition of inspiration; they claimed that in animals with this pontile apparatus extirpated, blocking of the vagi results in apneusis. Although it is generally agreed that the pneumotaxic center is located in the upper few millimeters of the tegmentum of the pons, direct stimulation of the pontile structures have given no obvious indications of the presence of this center. It is important to mention that the evidence for the existence of a pontile pneumotaxic mechanism has been based entirely upon complete or partial transection experiments, and that the accuracy of such procedures, in delimiting neuronal boundaries, is always questionable. Indeed, a number of workers (16, 17) have reported a modified type of rhythmic breathing in animals after pontile ablation and bilateral vagotomy. This is explained by Pitts (18) as attributable to remnants of the pneumotaxic center extending downward into the medulla. The topography of the spasmodic respiratory center in the myelencephalon, and the repetitive character of the response suggest that it may be related to the mechanism of pneumotaxis and may be a factor responsible for the residual breathing in the vagotomized and pontile transected animals.

## SUMMARY

The brain stem of the cat was stimulated with the aid of the Horsley-Clarke stereotaxic instrument and a spasmodic respiratory response which is recognized as coughing, sneezing and retching has been elicited (figs. 1-4). The central mechanism regulating this response has been localized in the dorsolateral region of the myelencephalon including the structures corresponding to the descending vestibular tract and nucleus, the tractus solitarius and nucleus, and the vagal and glossopharyngeal rootlets (fig. 5). Stimulation with low frequency (less than 40 impulses per second) and moderate voltage currents favor the occurrence of the spasmodic response (figs. 2 and 3).

A possible relationship of the spasmodic respiratory center to the mechanism of pneumotaxis is discussed.

The author wishes to express his appreciation to Dr. S. C. Wang for the invaluable suggestions given throughout the course of this study.

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# ACTION POTENTIALS IN RAT MUSCLE WITH TWITCH TENSION POTENTIATED BY KCl TREATMENT, ADRENALECTOMY, TETANUS AND TREPPE<sup>1</sup>

SHEPPARD M. WALKER

*From the Department of Physiology, Washington University, School of Medicine*

ST. LOUIS, MISSOURI

IN A previous study (1) it was found that the response of rat muscle to single stimuli showed a greater developed tension and a longer contraction time in adrenalectomized animals than in normal controls. The increased contraction time and the prolonged action potentials observed after adrenalectomy led to the suggestion that repetition might account for a part of the increased tension. In the same report it was shown that the response of muscle potentiated by intraperitoneal injection of KCl had a longer contraction time than the response of untreated muscle.

Brown (2) studied the effect of K on the action potential in muscle of the cat after intra-arterial injection of KCl. This investigator observed a decrease in height of the action potential but he did not report a change in duration of the action potential or an increase of contraction time of the KCl-treated muscle.

In a recent report (3) it was shown that the increased tension in responses of muscle to single stimuli in rats treated with desoxycorticosterone acetate is accompanied by repetitive action potentials. However, these potentiated responses differ from those seen after KCl treatment, adrenalectomy and tetanus in that they disappear after the third to sixth response to single stimuli.

The present study was undertaken to determine the nature of the action potentials which accompany the responses to single stimuli in muscle showing increased twitch tension as a result of KCl treatment, adrenalectomy, tetanus or treppe. A preliminary report on this study has been made (4).

## METHODS

The records were obtained under ether anesthesia from immature male rats weighing 150 to 200 gm. Action potentials were recorded from the gastrocnemius muscle with a cathode ray oscillograph. Mechanical records of muscle contraction were made simultaneously using an isometric lever and optical recording as previously described (1). The cut sciatic nerve was stimulated with brief shocks three to four times threshold strength. Normal rats were injected intraperitoneally with 40 to 80 mg. of KCl per 100 gm. of body weight. The records were obtained from adrenalectomized rats after the appearance of signs of severe adrenal cortex insufficiency, i.e., weight loss and muscular weakness. A few adrenalectomized animals were allowed to drink one per cent KCl solution instead of tap water. Tetanus was produced by repetitive shocks at the rate of 200 per second for one to 10 seconds. Treppe was brought about by single shocks at the rate of one per second. As a basis of comparison, control records were made from rested muscle of normal rats.

To obtain blood for analyses, the heart was exposed and artificial respiration was given. The

Received for publication June 21, 1948.

<sup>1</sup> Aided by a grant from the U. S. Public Health Service.



blood was drawn from the left ventricle into a syringe moistened with heparin. The plasma was separated immediately by centrifugation.

Plasma and muscle K were determined by the potassium phosphotungstate method of Van Slyke and Rieben (5). This method was modified for determination of muscle K. The muscle was placed in a platinum crucible and dried to constant weight in an electric oven at  $105^{\circ}\text{C}$ . About 0.5 cc. of 4 N  $\text{H}_2\text{SO}_4$  per gm. of muscle was added and the crucible was placed under an Infra Radiator for about an hour. The crucible was then transferred to a cold muffle furnace and the muscle was ashed at  $480$  to  $500^{\circ}\text{C}$ . The ash was dissolved in 1 to 2 cc. of water containing one drop of concentrated HCl. The solution was transferred to a 10 cc. volumetric flask containing a drop of phenolphthalein indicator and was made up to volume.  $\text{Ca}(\text{OH})_2$  was added until the solution became slightly alkaline. The solution was then left over night and was filtered the next day. Further dilutions were made depending on the size of the muscle. For 1- to 2-gm. muscles a one to 100 dilution was used. Aliquots of 3 cc. were taken for analyses. For determination of muscle Na, 3 cc. aliquots of the ashed muscle were determined gravimetrically as the uranyl zinc acetate by the Butler and Tuthill (6) method. Plasma Cl was determined by the Sendroy (7) method. The powdered muscle for Cl determination was mixed with five times its weight of sodium carbonate and ashed at  $450^{\circ}\text{C}$ . The ash was dissolved with warm water made slightly acid with nitric acid and Cl was determined with the Volhard titration.

The extracellular water was calculated from the ratio of muscle Cl to the concentration of Cl in the plasma water. It was assumed that all muscle Cl is extracellular. The water content of the plasma was considered 94 per cent. The calculations used to obtain the data shown in table 2 were essentially the same as those described by Hastings and Eichelberger (8) and by Lowry and Hastings (9).

## RESULTS

*KCl treatment.* In the first series of experiments action potential records were obtained with both recording electrodes placed in the belly of the gastrocnemius. The leads in this position have been found more effective than the belly and tendon leads for detection of repetitive discharges involving only a small portion of the muscle fibers (3). Results typical of the findings in 5 rats are shown in figure 1A in the record taken 40 minutes after the intraperitoneal injection of 80 mg. of KCl per 100 gm. of body weight. Although the mechanical response which accompanied this record showed a 60 per cent increase of twitch tension no repetitive discharges were seen.

The decrease in height and increase in duration of muscle action potential produced by prolonged treatment with KCl are marked when one lead is placed in the belly of the muscle and the other lead is placed in the tendon or in a distant point (fig. 1B). The gradual decrease in height and increase in duration of action potential are shown in the records in figure 1C. These changes in electrical response were accompanied by progressive increases of contraction time and twitch tension. The percentage increase of twitch tension was approximately equal to the percentage increase of contraction time in the KCl-treated muscle. For example, the mechanical response recorded 55 minutes after KCl injection (fig. 1C) showed about 65 per cent increase in contraction time and about 70 per cent increase in twitch tension.

When belly and tendon or belly and distant leads were employed, action potential records were rather smooth before KCl treatment if the belly lead were placed near the surface of the muscle (fig. 1B). Multiple peaks frequently appeared in the electrical records, however, when the mechanical responses of the muscle were potentiated by K (fig. 1B). By placing the belly lead in the interior of the muscle it was

possible to obtain action potential records with multiple peaks from the untreated muscle. These peaks, or components, showed temporal dispersion after KCl treatment (fig. 2, A and B). The dispersion was not uniform, however, because the peaks tended to overlap and partially lose their identity. The latent period between the stimulus and the beginning of the action potential was two to three times longer after prolonged KCl treatment (fig. 2A).

The relationship between decreases of action potential height and increases of action potential duration was not linear. The height of the action potentials diminished *more* rapidly during the first 20 minutes after KCl injection and less rapidly as potentiation of muscle tension progressed. On the other hand, the duration of the action potentials increased *less* rapidly during the first 20 minutes and more rapidly during the subsequent period. These changes are shown in A and B of figure 3

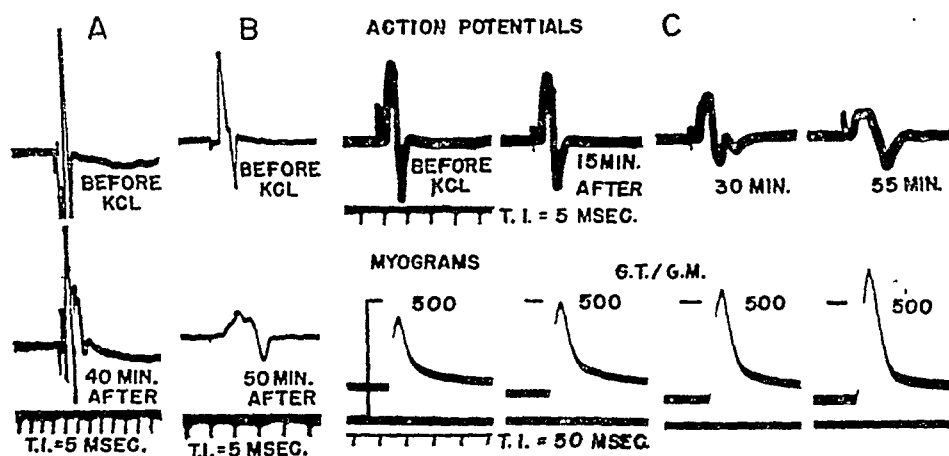


Fig. 1. EFFECTS OF INTRAPERITONEAL INJECTION of 80 mg. of KCl per 100 gm. of body weight on the muscle action potential and twitch tension of the gastrocnemius of the rat. The muscle was stimulated with single indirect shocks 3 to 4 times threshold strength. A. Action potentials with both leads in the belly of the muscle. B. Action potentials with one lead in the belly of the muscle and the other lead in a distant point of the animal. C. Action potentials (above) with belly and tendon leads and corresponding mechanical records of twitch tension (below).

which is a diagram of the average and range of responses induced in 3 different animals. It is interesting to note that the marked diminution in height of action potential during the first 20 minutes of KCl treatment is similar to that obtained by Brown (2) in the cat when intra-arterial injections of KCl were made.

Marked increases of plasma K were found 15 minutes after KCl injection (table 1). Slight increases of muscle K (table 1) and intracellular concentration of K (table 2) were induced by 15 minutes of KCl treatment. Application of the data given in table 2 shows that consideration of changes in cell weight may account for 70 per cent ( $118 \times 900/874 = 121.5$ ) of the increase of intracellular K. On the basis of changes in intracellular water 75 per cent ( $160 \times 664/639 = 166$ ) of the increase of intracellular concentration of K may be due to dehydration of the cells. The level of plasma K found in animals sacrificed about one hour after injection of KCl was slightly higher than that seen 15 minutes after KCl injection. Moreover, a greater increase of intracellular K was observed in the muscle of animals treated for one hour with KCl. The data in table 2 indicate that about 50 per cent of the in-

crease in intracellular K concentration, in muscle treated for one hour, is due to movement of water out of the cells. The percentage increase of extracellular K concentration greatly exceeded the percentage increase of intracellular K concentration in all muscle treated with KCl (table 2). Plasma and muscle Cl increased and plasma and muscle Na decreased during KCl treatment. Although the total water content of muscle was unchanged (table 1), the intracellular water was decreased and the extracellular fluid was increased by KCl treatment.

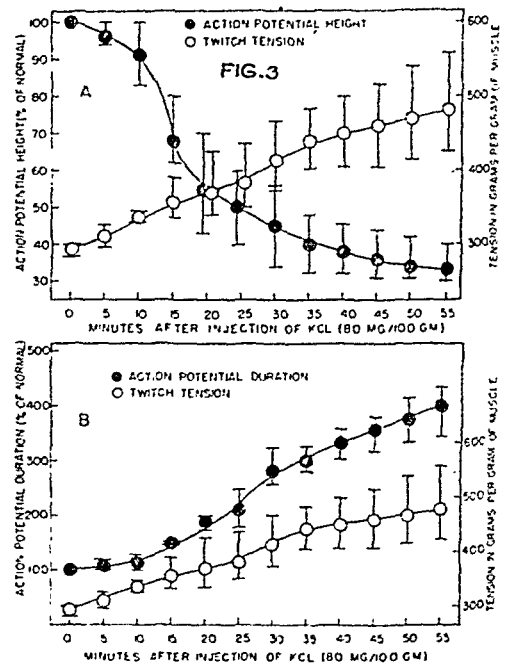
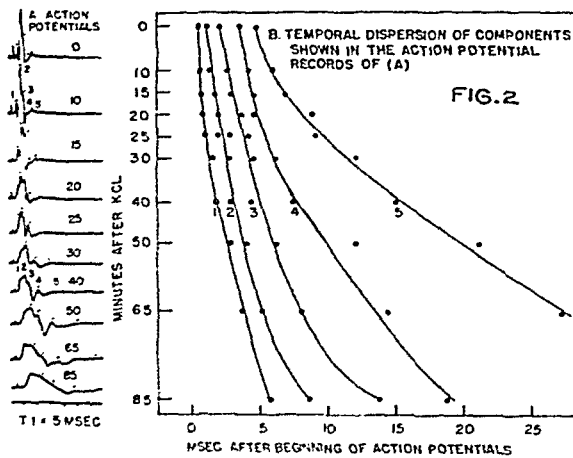


Fig. 2. EFFECTS OF INTRAPERITONEAL INJECTION of 80 mg. of KCl per 100 gm. of body weight on the gastrocnemius muscle action potential with one lead electrode placed in the center (*interior*) of the belly in such a position that several discreet points (1-5) were seen on the records. The other lead was in the tendon. The muscle was stimulated with single indirect shocks 3 to 4 times threshold strength. A. Action potentials with the time of recording indicated as minutes after KCl injection. B. Graph showing the temporal dispersion of the components in the action potentials shown in A. (The twitch contraction time increased from 18 to 32 msec. and the twitch tension increased 80 per cent during the progress of the experiment.)

FIG. 3. DIAGRAMS SHOWING THE RELATION OF TWITCH TENSION to action potential height (A) and action potential duration (B) in 3 rats injected with 80 mg. of KCl per 100 gm. of body weight. The circles show averages and the horizontal bars show the ranges of values.

*Effects of adrenalectomy.* It has been found that the muscles of adrenalectomized rats develop tension 40 to 50 per cent greater than that seen in normal muscle in response to single stimuli (1). The muscle action potentials obtained with belly to tendon leads in the adrenalectomized animals show an increased duration when compared with the action potentials from similarly placed leads in normal muscle. These experiments were repeated in 5 animals showing severe adrenal insufficiency and the previous results were confirmed (fig. 4, A and B). Although the height of the action potentials in both adrenalectomized and normal animals varied considerably, presumably depending upon the position of the lead electrodes with respect to the adjoining muscle fibers, the duration of the action potentials was less variable and it was

consistently greater in the adrenalectomized than in the normal animals. In an attempt to exaggerate the increase of potassium in adrenalectomized animals 1 per cent of KCl was given in the drinking water. The plasma and intracellular K levels and the duration of the muscle action potentials (figure 4C) were increased by the

TABLE 1. AVERAGE VALUES FOR ANALYSES OF RAT MUSCLE AND PLASMA

NO. OF RATS	MUSCLE (PER KG. OF FRESH TISSUE)				PLASMA (PER LITER)		
	water	K	Na	Cl	K	Na	Cl
<i>KCl treatment<sup>1</sup> for 15 minutes</i>							
3	gm. 765 ± 0.9 <sup>2</sup>	mM 109.3 ± 0.4	mM 16.3 ± 0.6	mM 15.7 ± 0.7	mM 13.4 ± 0.7	mM 144 ± 0.7	mM 113 ± 3.2
<i>KCl treatment for 55 to 65 minutes</i>							
3	764 ± 1.0	112.7 ± 1.8	14.1 ± 0.9	16.0 ± 1.1	14.8 ± 0.6	142 ± 1.2	118 ± 0.4
<i>Control rats</i>							
4	763 ± 1.1	106.5 ± 0.8	17.2 ± 0.6	12.0 ± 0.5	4.5 ± 0.2	150 ± 2.3	110 ± 1.3

<sup>1</sup> 80 mg. of KCl (2%) per 100 gm. of rat were injected intraperitoneally.

<sup>2</sup> Standard error of the mean =  $\sqrt{\frac{\sum d^2}{n(n-1)}}$ .

TABLE 2. AVERAGE VALUES FOR DISTRIBUTION OF WATER AND POTASSIUM IN MUSCLE. DATA DERIVED FROM TABLE 1

NO. OF RATS	EXTRACELLULAR FLUID	TOTAL CELL WT. <sup>1</sup>	INTRACELLULAR WATER		K	K	K
	Per kg. muscle	Per kg. muscle	Per kg. muscle	Per kg. of cells	Per kg. extracellular water	Per kg. of cells	Per kg. intracellular water
<i>KCl treatment for 15 minutes</i>							
3	126 ± 3.3	874 ± 3.3	639 ± 4.2	732 ± 1.9	13.5 ± 0.77	123 ± 0.6	168 ± 0.9
<i>KCl treatment for 55 to 65 minutes</i>							
3	130 ± 2.9	870 ± 2.9	635 ± 4.4	730 ± 2.1	14.9 ± 0.65	128 ± 2.9	174 ± 3.1
<i>Control rats</i>							
4	100 ± 4.1	900 ± 3.2	664 ± 3.8	737 ± 0.9	4.52 ± 0.34	118 ± 1.4	160 ± 2.0

<sup>1</sup> The values in this column include all extracellular material (i.e., collagen and elastin) not calculated with extracellular fluid.

drinking of KCl solution. However, the developed tension was about equal to that observed in adrenalectomized animals receiving tap water. A study in 5 adrenalectomized animals with both leads placed in the belly of the muscle showed single muscle action potentials in response to single shocks.

*Effects of tetanus.* Post-tetanic responses of normal muscle stimulated with

200 shocks per second for two seconds or less were accompanied by action potentials with reduced height and unchanged duration. The responses after tetanic stimulation for three seconds showed action potentials with further decrease in height and with slight increase of duration (fig. 5A). Further increases of the period of tetanic stimulation gradually decreased the height and increased the duration of post-tetanic action potentials. A marked increase of duration was obtained by 10 seconds of tetanic stimulation (fig. 5B). The time required for return to action potentials similar to the pretetanic ones did not appear to be related to the duration of tetanus.

Fig. 4. EFFECTS OF ADRENALECTOMY and of adrenalectomy plus a one per cent KCl drinking solution on the action potentials and twitch tension. *A*. Comparison of action potentials and twitch tension in the gastrocnemius muscle of normal ( $\times$ ) and adrenalectomized (*A*) rats. *B*. Comparison of action potentials in the gastrocnemius muscle of normal ( $\times$ ) and adrenalectomized-KCl-treated (*A* + 1% KCl) rats. The action potential records were made with belly and tendon leads and they are superimposed.

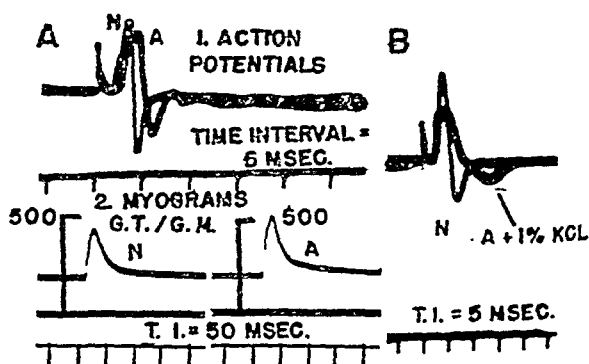
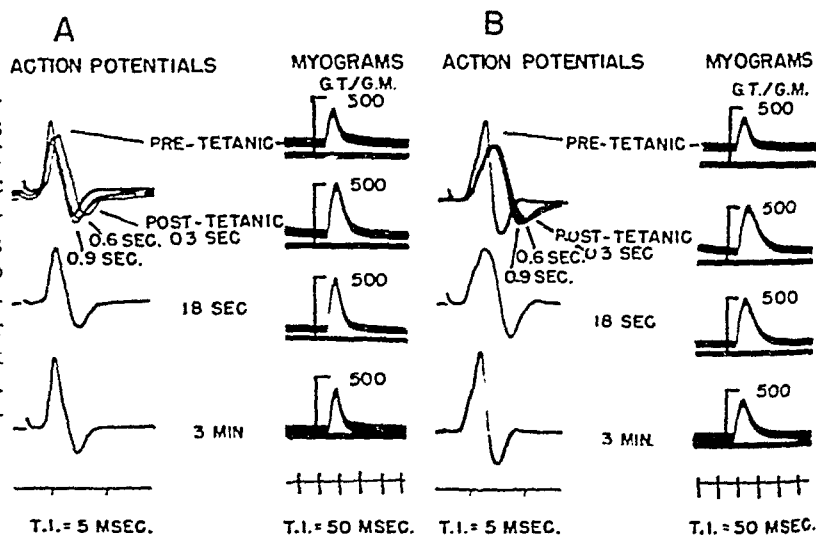


Fig. 5. ACTION POTENTIALS AND MYOGRAMS showing the effects of tetanus on post-tetanic responses. *A*. Responses before and after 3 seconds of tetanus at 200 shocks per sec. *B*. Responses before and after 10 seconds of tetanus at 200 shocks per second. Stimulation was indirect.



For example, the time for recovery of the pretetanic form of the action potential was approximately 3 minutes both for a 3-second tetanus and for a 10-second tetanus (fig. 5, A and B). The marked increases of action potential duration observed after prolonged tetanic stimulation were not accompanied by proportional increases of post-tetanic potentiation of tension. In other words, maximal potentiation was attained by three to five seconds of tetanic stimulation. The contraction time and the relaxation time in post-tetanic twitches were either less than or about equal to those seen in pretetanic twitches up to the point of maximal potentiation. Prolongation of the period of tetanic stimulation beyond the point of maximal potentiation induced greater contraction times and slower relaxation rates in post-tetanic responses than those found in pretetanic responses.

Post-tetanic responses of muscle 30 minutes after intraperitoneal injection of KCl showed further decreases in height and increases in duration of action potentials. The peak twitch tension attained by tetanus after KCl treatment was greater than that attained by tetanus alone. No evidence of repetition was seen in post-tetanic responses of normal or of KCl-treated rats.

*Effects of treppe.* There is no significant change in either height or duration of action potentials in responses potentiated by single shocks at one per second. Brief periods of tetanic stimulation at the point of maximal potentiation of tension resulting from treppe brought about further increases of tension in the post-tetanic responses to single shocks. The action potential records of these post-tetanic responses showed increased duration and decreased height.

Muscles potentiated by intraperitoneal injection of KCl showed increases of twitch tension during treppe which were about equal to the increases seen in treppe of normal muscle. Since the muscle was already potentiated by K before the beginning of exercise the tension attained during treppe in KCl-treated muscle exceeded that obtained by treppe in untreated animals. The action potentials in the KCl-treated muscle underwent little change during treppe. It should be noted that they were already reduced in height and increased in duration before exercise was begun.

#### DISCUSSION

The findings in this study confirm the observations by Brown and Euler (10) and Feng and Li (11) that marked potentiation of muscle response may be obtained by KCl treatment in the absence of repetitive discharges. The twitching which may occur during close intra-arterial injection of KCl (12, 13) is independent of the potentiated twitch responses to single shocks. The observation that the preponderance of K excess is extracellular throughout the period of KCl treatment (tables 1 and 2) suggests that action of K on the membrane of the muscle fibers may play an important rôle in bringing about 1) increased duration and decreased height of the action potential, 2) increased contraction time and 3) increased twitch tension in response to single shocks.

The changes in positions of the multiple peaks in the action potential, recorded in figure 2A and shown diagrammatically in figure 2B, indicate that dispersion in time of these components contributes to the change in form of the action potential during the progress of KCl treatment. Two factors may be involved in the temporal dispersion of fiber responses in indirectly stimulated muscle: *a*) unequal delay in initiation of response of different muscle fibers and *b*) decreased conduction rate. The increase of the latent period between the stimulus and the beginning of the action potential showed that the start of the response of all fibers is delayed by KCl treatment. The delayed beginning of the endplate potential in frog muscle treated with KCl (14) suggests that the increased latent period between stimulus and action potential is due to an action of K at the neuromuscular junction. It is obvious that the height would be reduced and the duration would be increased in the composite action potential to the extent that the delay of response of the component muscle fibers is unequal. The appearance of multiple peaks in the action potentials of muscle potentiated with KCl (fig. 1B) is regarded as evidence that the response of a part of the muscle fibers is delayed more than that of other fibers.

Granting that the conduction distance between points of excitation and points of recording is unequal in the various muscle fibers, a decrease of conduction rate would result in dispersion of the components of the action potential and thus bring about a decrease in height and an increase in duration of the composite action potential. Recently, Brown *et al.* (15) have shown that addition of adrenalin to a bath containing a phrenic-diaphragm preparation of the rat induced an increase in duration of the muscle action potential, which they attributed to a delay of the spread of the excitation wave in the individual muscle fibers. An earlier report (16) is of particular interest in connection with the present study because it was shown that the potentiating effect of adrenalin on the diaphragm preparation is dependent upon reduction of the K content of the bath fluid.

On the basis of the discussion thus far no change in the height or the duration of the gross action potential would be expected in the absence of dispersion of the components contributing to the action potential. However, two possible effects of K remain which might alter the form of the action potential independently of dispersion. A decrease in rate of depolarization and repolarization would increase the duration of the action potential. Furthermore, a decrease of the resting potential by K excess, as has been shown by many reports, could conceivably result in a decrease in height.

With the exclusion of repetition as a factor only two effects remain which may account for the increased contraction time of K potentiated twitch responses: *a*) dispersion in time of the response of the different fibers and *b*) prolongation of contraction time of the individual fibers contained in the muscle. Moreover, to account for the increase of twitch tension resulting from KCl treatment two assumptions may be made: *a*) the contraction time of the muscle is increased and *b*) the contractile strength of the muscle fibers is increased. Obviously any dispersion of response of component muscle fibers would result in a decrease of twitch tension provided the contraction time of individual fibers remained constant. On the other hand, any increase of contraction time of the individual muscle fibers would bring about an increase of twitch tension, provided a given amount of dispersion existed, because the fibers responding early would allow more effective summation with the contraction of fibers responding late.

The view that K increases the contraction time of muscle fibers is attractive because it offers an explanation, at least in part, for the observed increase both of tension and of contraction time in the potentiated twitch. Furthermore, this view lends itself to the implication of an extracellular action of K on the membrane. Kuffler (17) has shown that K and veratrine contractures last for the duration of the membrane changes which they induce. He suggested that the essential condition for initiation of processes in the contractile mechanism is the 'removal' of at least a part of the membrane and that any process restoring the membrane will also cause relaxation. The duration of membrane 'breakdown' during a single response of a KCl-treated muscle may be regarded as increased, if it is assumed that conduction is slowed and that the depolarized area is not decreased. Any increase of the depolarized area would further increase the duration of membrane 'breakdown'. If the interpretations regarding contractures are extended to apply to contractions, it is plausible to suggest that the contraction time of a muscle fiber may be increased by increased duration of membrane 'breakdown' resulting from passage of the excitation wave.

The author is not aware of any experimental evidence to support the suggestion by Brown and Euler (10) and Walker (1) that at least a part of the potentiating effect of K on twitch tension is due to increased contractile strength, presumably involving an action of K on the muscle fiber substance. The present study does not rule out intracellular action of K but it does suggest an extracellular potentiating effect.

The above observations, that in KCl-treated muscle there is increase of twitch tension, increase of twitch peak time and increase in duration of action potential, are readily brought into harmony with the hypothesis offered by Gilson *et al.* (18). Their treatment of the tension-time course for the twitch assumed instantaneous mobilization of an increment of material as a first step in a series of chain reactions. If activation of KCl-treated muscles is such that the process of activation or mobilization is not instantaneous but occurs over a significant period of time and, at least for purposes of argument, proceeds at constant rate, there would result an increase of contraction time and an increase of peak tension for the response to a single shock. The form of the twitch response would thus resemble that of a very brief tetanus.

Fenn and Cobb (19) found that tetanic stimulation brings about a decrease of K in the stimulated muscle of the rat. It was later shown (20) that tetanic stimulation of muscle in the cat produces an increase of plasma K. The decreased action potential height and increased action potential duration seen after tetanus (fig. 5) offered additional support for the view that a preponderance of K outside of the cells may be important in bringing about these changes in action potential. Also in this connection it should be pointed out that in a muscle already potentiated by K the characteristic changes of the action potential induced by K are exaggerated by tetanus.

The absence of significant changes of the action potentials during stimulation at one shock per second suggested that at this frequency of response K does not accumulate in appreciable quantity in the extracellular fluid immediately surrounding the muscle fibers. There is apparently a graded effect upon the action potentials which is dependent upon both frequency and duration of stimulation. For example, tetanus produced with a stimulus frequency of 200 per second induced a slight decrease of action potential height and no change of action potential duration in post-tetanic responses of muscle stimulated for one to two seconds. Tetanic stimulation for two to three seconds at the same frequency induced greater decrease of action potential height and some increase of action potential duration (fig. 5A). Tetanic stimulation for 10 seconds brought about a marked decrease of action potential height and a marked increase of action potential duration (fig. 5B).

The prolonged muscle action potential in adrenalectomized rats (fig. 4A) confirmed the earlier findings (1), but they did not support the view that repetition is involved with the large mechanical responses to single shocks in these animals because none of the leads employed showed any evidence of repetitive discharges distinguishable from the initial action potential. It is now believed that the changes found in the muscle action potentials of adrenalectomized rats may be due, at least in part, to increases of the intracellular and extracellular concentration of K. Increased mechanical response to single shocks is perhaps related to changes in concentration of K.

#### SUMMARY AND CONCLUSIONS

Action potential records with various positions of the lead electrodes were made in muscle with twitch responses potentiated by KCl treatment, adrenalectomy,



tetanus and treppe. The records of action potential showed no evidence of repetition in any of the potentiated responses although the increased peak twitch tension in muscle of KCl-treated and adrenalectomized animals was accompanied by increased contraction time. KCl treatment and tetanus induced a decrease in height and an increase in duration of muscle action potentials. Adrenalectomy brought about an increase in duration of action potential records. Because the height of the action potential is dependent upon the number of muscle fibers contributing to the action potential no conclusions were drawn with regard to height, although the height was usually smaller when compared with action potentials of normal muscle having similarly placed leads. No significant change in the action potential records was found in responses potentiated by treppe induced by single shocks at 1 sec.

Possible effects of K on asynchrony of response, on conduction rate and on the rate of depolarization and repolarization were considered. The relation of these effects to changes in the action potential records was discussed. It was suggested that an increase of extracellular K may play an important rôle in bringing about the decrease in height and the increase in duration of the muscle action potentials. It was pointed out that the slight increase of intracellular K in the KCl-treated muscle is accompanied by a much greater increase of extracellular K. It was further noted that tetanus increases plasma K (20) and that KCl treatment induces changes in the muscle action potential similar to those seen after tetanus. It was suggested that K excess may increase the duration of membrane 'breakdown' and thus increase the contraction time and the strength of contraction of muscle fibers. The possibility that an intracellular action of K in excess might increase the contractile strength of the muscle fiber substance was not excluded.

The author is indebted to Marian Schmied for the chemical analyses and for technical assistance.

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# BRAIN METABOLISM IN EMOTIONAL EXCITEMENT AND IN SLEEP

D. RICHTER AND R. M. C. DAWSON

*From the Neuropsychiatric Research Centre, Whitchurch Hospital*

CARDIFF, SOUTH WALES

EVIDENCE has been obtained that the metabolic activity of the brain is increased in convulsions, trauma, anoxia and shock. This is indicated by the rise in lactic acid and decrease in high-energy phosphorus compounds found in the brains of animals killed under these conditions. Conversely, there is a fall in the lactic acid in the brain and an increase in labile phosphate in animals killed under anesthesia (1-3). The present investigation was carried out to test if evidence could be found of similar biochemical changes in the brain in sleep and in emotional excitement under normal physiological conditions.

## METHODS

Lactic acid estimations were carried out on the whole brains of young Wistar albino rats of 30 to 40 gm. The animals were killed by immersion in liquid air, which produced a rapid fixation of any biochemical changes in the tissues and limited the formation of lactic acid in the brain by post-mortem glycolysis.

*Method of fixing with liquid air.* Stone (1) found it took 2 to 3 seconds to freeze the brain of a mouse, but Le Page (3) found it took about 40 seconds to freeze completely with liquid air the abdominal organs of a 300-gm. rat. In view of the rapidity of post-mortem changes in the brain, the rate of freezing was reinvestigated with rats of the size used in the present work. Experiments with a calibrated thermocouple inserted into the cranial cavity of 35-gm. rats showed that the surface of the cortex fell to 0°C. in 4 to 5 seconds on immersion of the whole animal in liquid air. With the thermocouple in the deeper parts of the brain the temperature remained practically stationary at 37°C. until a sudden drop in temperature occurred, bringing it below 0°C. in 9, 20, 9 and 16 seconds in four consecutive experiments. While it clearly took several seconds for the wave of freezing to spread from the surface to the interior of the brain, it is unlikely that the circulation of the central parts of the brain stopped immediately or that true post-mortem changes occurred in the interior of the brain throughout the whole of this period. This view is supported by the work of Kerr (4), who found that satisfactory phosphagen figures can be obtained by applying liquid air to the skulls of young cats, in which the process of freezing the brain must take much longer. The observed lowering of the brain lactic acid in sleep, which was found in the present investigation, gave further evidence that post-mortem changes during the process of freezing were probably not extensive with the technique employed.

In obtaining normal values the rats were transferred rapidly from their cages into the liquid air, as any delay in this procedure tended to frighten them and caused the brain lactic acid level to be raised. Killing by decapitation and subsequent freezing of the heads was found to be less satisfactory as it gave much higher lactic acid figures for normal animals: in three experiments the brain lactic acid figures obtained by this method were 43, 37 and 47 mg. per cent when the heads were transferred to liquid air in 2, 3, and 15 seconds respectively after decapitation. Kerr also found autolytic changes more marked after decapitation than when the brain was frozen in the whole animal. The reason may be that with the circulation cut off the brain rapidly becomes anoxic and glycolysis proceeds in the interior during the period of freezing through from the surface.

*Lactic acid estimation.* After careful dissection the frozen brains were powdered in a previously cooled crusher and the powder was transferred to weighed centrifuge tubes containing 4 ml. zinc sulphate solution at 0°C. as described by Blatherwick, *et al.* (5). The method of estimation was that of Friedemann, Cotonio and Shaffer (6), with the modifications and improvements introduced by Friedemann and Graeser (7), Edwards (8) and Stone (1). Although more laborious, this method was found to give more accurate and more reliable figures than the colorimetric methods of Miller and Muntz (9) and Barker and Summerson (10). The distillation apparatus was similar to that used by Stone (1), but made all in one piece and therefore containing no joints, tap-grease or rubber connections. Talc powder was found more satisfactory than glass beads to prevent bumping. The use of the copper hydroxide precipitation made the method very specific for lactic acid and recovery tests with a standard solution of recrystallized lithium lactate, which were repeated with each experimental series, gave recoveries within 3 per cent of the theoretical with quantities down to 0.1 mg. lactic acid.

## RESULTS

*Normal series.* In a preliminary series of 10 normal rats taken in the resting state the brain lactic acid level ranged from 13.4 to 24.4 mg. per cent with a mean of 18.8 (table 1). This figure agreed with the mean of 18.9 mg. per cent for mouse brain found by Stone (1), who also found a wide range of individual values ranging from 12 to 23 mg. per cent.

*Brain lactic acid in sleep.* The animals comprising the normal series were mainly littermates of almost identical size and weight and in looking for an explanation for the wide individual variation in the brain lactic acid level, it was noted that lower values were generally given by animals which were dozing at the time when they were transferred to the liquid air, while higher values were obtained with animals which were wide awake. The investigation of the brain lactic acid in sleeping animals met with considerable difficulty until it was found that deep sleep could be induced by leaving the rats for a time in strong sunlight. Artificial sunlight was equally effective: Dozing animals opened their eyes at once when touched, but animals in deep natural sleep did not open their eyes or appear to wake up in the brief period of less than a second required to transfer them from their cages into liquid air. This was therefore taken as the criterion of sleep. A series of 6 rats taken in the sleeping state showed

less individual variation in the brain lactic acid and a mean level of 12.2 mg. per cent, which was considerably lower than that of the normal series. The difference was statistically significant ( $P < 0.01$ ) when tested by Fisher's 't' test (table 2). The mean for sleeping animals approached the mean of 9.8 mg. per cent lactic acid found for a group of rats lightly anesthetized for a period of 8 to 30 minutes with nembutal.

TABLE 1. LACTIC ACID CONTENT OF BRAIN IN NORMAL RATS

RAT NO.	WT. OF RAT	LACTIC ACID	REMARKS
	gm.	mg. %	
1	36	16.2	Dozing
2	38	16.7	Dozing
3	40	20.1	Awake; resisted handling
4	48	17.9	Resting; slight movement
5	43	24.4	Quiet
6	31	22.6	Moving
7	31	22.3	Awake; resisted handling
8	25	15.5	Dozing
9	23	13.4	Quiet
10	40	19.1	Quiet; resisted handling
MEAN.....		18.8	

Recovery of lithium lactate standard 98%.

TABLE 2. LACTIC ACID CONTENT OF RAT BRAIN IN SLEEP AND IN ANESTHESIA

A. SLEEP		B. ANESTHESIA		
Rat no.	Lactic acid	Rat no.	Period of anesthesia	Lactic acid
	mg. %		min.	mg. %
1	12.3	7	8	13.2
2	10.3	8	8	7.7
3	11.9	9	15	8.0
4	10.8	10	15	4.8
5	13.9	11	30	15.4
6	14.1			
Mean.....	12.2			9.8

Recovery of lithium lactate standard 98%. Brain lactic acid in normal rat included in this series 17.5 mg. %. Period of sleep about 30 min. Anesthesia obtained by intraperitoneal injection of nembutal 50 mg/kg.

*Effect of emotional excitement.* Of the various methods of producing emotional excitement which were tried, the simplest was that of repeatedly removing their support by allowing them to drop from side to side in a glass beaker. There was a good deal of individual variation in their response to this treatment and some animals were less disturbed by it than others. Often there was a latent period of up to half a minute in which they showed little reaction, but finally they all gave objective evidence of fear, as by urinating, defecating and looking frightened. A few of them

made vigorous muscular movements and tried to jump out of the vessel, but more often they remained perfectly still and gave up trying to right themselves, so that little muscular exercise was involved. There was probably an element of anger as well as fear in their emotional state, for some of the rats were ready to bite when in this condition; but it may be doubted whether the emotional reactions of the rat can be accurately described in terms which are mainly applicable to man.

Estimations of the lactic acid content of the brains of rats taken after being frightened for  $1\frac{1}{2}$  to 4 minutes by the method described gave consistently higher values than those of normal littermate controls which were examined at the same time. In

TABLE 3. EFFECT OF EMOTIONAL EXCITEMENT AND EXERCISE ON THE LACTIC ACID CONTENT OF THE RAT BRAIN

A. FRIGHTENED		B. FRIGHTENED AFTER TUBOCURARINE		C. MUSCULAR EXERCISE	
Rat no.	Lactic acid	Rat no.	Lactic acid	Rat no.	Lactic acid
	mg. %		mg. %		mg. %
1	47.2	12	34.5	17	13.9
2	24.2	13	36.5	18	15.2
3	45.7	14	29.4	19	13.9
4	36.7	15	37.4	20	15.0
5	40.2	16	40.7	21	15.4
6	34.8			22	13.0
7	34.0				
8	23.7				
9	32.2				
10	50.3				
11	43.5				
Mean . . . . .	37.5		35.5		14.4

a) Period of frightening  $1\frac{1}{2}$  to 4 min. b) Rats given tubocurarine and after about 5 min., when the muscles were relaxed, frightened for 2 to 3 min. The blood lactic acid in 3 rats decapitated after treating them in the same manner was 17.8, 22.8 and 18.8 mg.%. All blood samples were taken from the carotid artery after decapitation. c) Exercise was vigorous running for 4 min. Recovery of lithium lactate standard 99%.

the frightened animals the lactic acid content ranged from 24.2 to 50.3 mg. per cent with a mean level of 37.5 for a series of 11 animals. The difference was statistically significant ( $P < 0.01$ ), (table 3).

Experiments in which the period of emotional excitement was varied showed that the rise in brain lactic acid in emotional excitement was a rapid process (fig. 1), which must correspond to a relatively high rate of metabolic activity in the brain. When the excitation was discontinued, the brain lactic acid soon came back to normal again and normal values were generally obtained within five minutes after discontinuing the stimulus. The rise in the brain lactic acid appeared to be a transient effect and there was a good deal of individual variation in the rate of rise and fall, as some animals were more easily frightened and remained frightened longer than others.

*Effect of muscular exercise.* As far as could be judged from simple observation, the rise in brain lactic acid in the previous experiments corresponded closely with the

degree of emotional excitement and showed no relation to the muscular activity observed in a number of the animals; but since it is known that muscular exercise can cause a rise in the blood lactic acid level, the effect of muscular exercise on the brain lactic acid required careful investigation. The blood lactic acid level is 12 to 18 mg. per cent in the normal rat. In 3 rats taken after four minutes of emotional excitement the blood lactic acid was found to have risen to 52, 47 and 67 mg. per cent; but, assuming the blood content of the brain to be approximately 5 per cent, the blood

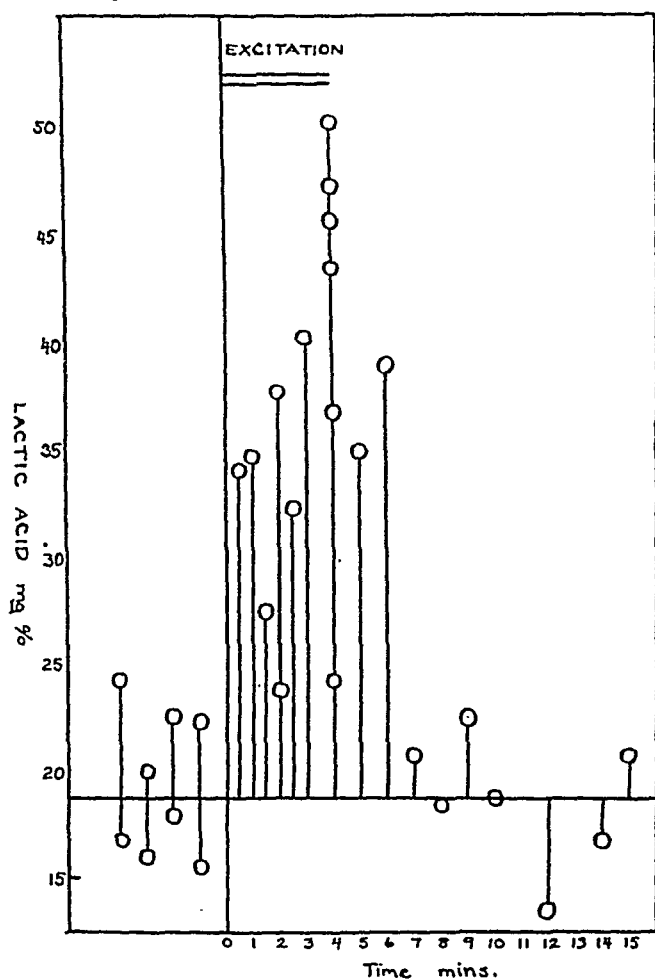


Fig. 1. SHOWING EFFECT OF EMOTIONAL EXCITEMENT on the brain lactic acid. The points on the left of the diagram are normal values, the horizontal line at 18.8 mg. % giving the normal mean. The points on the right of the diagram give lactic acid values for rats sacrificed at the times given on the time scale. The method of frightening was continued for 4 min., except for animals killed after a shorter period of frightening.

lactic acid could not nearly account for the mean level of 37.5 mg. per cent found in the brain. It was unlikely in any case that the brain lactic acid could have come from the blood, since the blood-brain barrier is relatively impervious to anions and it has been shown by Dameshek and Myerson (11) and by Stone (1) that lactic acid injected into the blood stream is not taken up significantly by the brain. This has recently been confirmed by Klein and Olsen (12), who showed in addition that the brain lactic acid is not increased by intravenous glucose, so that it was unlikely that emotional hyperglycemia had played any part. It was concluded that the brain lactic acid was formed in the brain and had not come from the blood.

The view that the rise in lactic acid in the brain in emotional excitement was not attributable to muscular activity was confirmed in a series of experiments in which

rats were excited after administering tubocurarine, so that muscular activity was practically abolished. The effective dose for this purpose was 0.1 ml. of a solution of 0.1 mg/ml. d-tubocurarine chloride per 40-gm. rat, given intraperitoneally. With this dose the respiration was not unduly embarrassed and the animals were not cyanosed, but muscular activity was greatly diminished. Emotional excitement produced the same rise in brain lactic acid in these animals, the figures ranging from 29.4 to 40.7 with a mean of 35.5 mg. per cent, although the blood lactic acid remained almost in the normal range (table 3). It therefore appeared that the rise in lactic acid in the brain in emotional excitement could not be attributed to the accompanying muscular activity.

In a series of further experiments designed to test directly the effect of muscular exercise alone on the brain lactic acid it was necessary to take special precautions to avoid emotional excitement, for it is hardly possible to induce untrained animals to take vigorous muscular exercise without exciting them. With this object in view a series of young rats weighing about 15 gm. were slowly conditioned to running for periods up to four minutes twice daily on an exercising wheel. After training in this way for eight days, by which time they weighed about 35 gm. they took their exercise without showing any signs of anxiety and indeed they appeared to enjoy it. Brain lactic acid estimations on a series of 6 trained rats after a period of four minutes vigorous running gave figures ranging from 13.0 to 15.2 with a mean of 14.4 mg. per cent. There was thus no evidence of any rise in the brain lactic acid in muscular exercise: the mean level after exercise was even somewhat below that of the normal series. This may be due to the effect of frequent handling in reducing their anxiety when handled.

#### DISCUSSION

It has been shown that the lactic acid content of the rat brain, analyzed after rapid fixation by freezing in liquid air, depends on the physiological state of the animal at the time. Rats taken in the sleeping state gave a significantly lower brain lactic acid content than controls in the normal waking state. Rats taken during emotional excitement gave a brain lactic acid level considerably higher than the normal and 300 per cent above the mean level for sleeping animals. Unless it is believed that these changes occurred in the brief period of freezing with liquid air, it must be concluded that they represent biochemical changes occurring in the brain *in vivo* under normal physiological conditions.

Muscular exercise appeared to play no part in the rise in lactic acid in the brain in emotion since *a*) the effect was still observed in animals immobilized by tubocurarine and *b*) no rise in the brain lactic acid occurred in muscular exercise alone without emotional excitement. The lactic acid content of the brain is not increased by adrenaline injection (1, 12) and the rise in lactic acid in the brain in emotion was observed in the absence of any significant rise of lactic acid in the blood. The simplest explanation of these observations is that in emotional excitement the increased functional activity of the brain is associated with increased glycolytic activity, involving a breakdown of high energy phosphorus compounds and the liberation of lactic acid. The changes in the brain would thus parallel those which occur in func-

tional activity in muscle. This view is supported by the work of Stone (1) and Le Page (3) on the changes in the brain produced by anesthetic and convulsant drugs. That biochemical changes occur in the brain in emotion is indicated by the changes in the electroencephalogram, as also by the chromatolysis in the nerve-cells in prolonged emotional excitement.

The results of the present investigation are in general agreement with the observations of Stone and of Le Page, but they suggest that the rise in brain lactic acid in exercise observed by Stone should be attributed rather to the emotional excitement, which is hardly avoidable when untrained animals are made to take strenuous exercise. Emotional excitement may contribute to the biochemical changes in the brain produced in some cases by drugs and in experiments such as those of Le Page on experimental shock. Gibbs *et al.* (14) reported that the brain normally liberates a small but significant amount of lactic acid into the blood, as shown by arterio-venous differences in experiments on man. This gives evidence of the formation of lactic acid in the brain under normal physiological conditions. The present work suggests that the rate of formation of lactic acid varies with the functional activity of the brain, being lowered in sleep and increased in emotional excitement.

#### SUMMARY

The lactic acid content of the rat brain is reduced in sleep and increased in emotional excitement. The rise in lactic acid in the brain in emotion is not due to concomitant muscular activity, since the effect was still observed in animals immobilized by tubocurarine. The brain lactic acid was not raised by muscular exercise in trained animals. The rise in lactic acid in the brain in emotion is a transient effect, followed by a rapid return to normal when the stimulus is discontinued.

The authors thank the Medical Research Council for a whole time grant to one of us (R. M. C. D.) and for a grant for expenses; the Rockefeller Foundation and the Royal Society for grants for equipment; Mrs. Y. Bezoari for her help in obtaining young rats; and Mr. J. Russell for valuable technical assistance.

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# INTRACRANIAL NOVOCAIN ANESTHESIA IN FROGS

BRUNO KISCH

*From the Physics Department, Mount Sinai Hospital*

NEW YORK CITY

**R**ECENTLY a method of anesthetizing fish (1) by the injection of 0.25 to 1.0 cc. of a 3 to 7 per cent novocain solution into the cranial cavity was described. Fish treated in this way are immobilized after a short excitation stage; the effect of the injection lasts from 30 to 60 minutes. They continue to breathe and if the head is kept submerged in a small seawater tank the heart can be used for a long time for experiments.

The usual way to immobilize or anesthetize frogs is either to use curare or to use the method of crushing the brain and spinal cord. This procedure causes a variable loss of blood. Therefore the use of intracranial novocain injections (1) was tried for frog experiments too. The method proved as effective here as in fish. For the experiments big bullfrogs were used, weighing between 250 and 500 gm. The solution of procaine hydrochloride (Novocain) used was between 3 and 5 per cent in strength. From 0.4 to 0.6 cc. of the solution was injected. The skull of frogs is harder to pierce than that of even a big selachian. Therefore somewhat stronger needles (20-gauge) were used. The preferred place for introducing the needle into the skull is caudally of the middle of a line connecting both eyes. The injection can also be made through the palate. In both methods the needle remained in some experiments in its place up to the end of the experiment to make sure afterwards that its apex was really in the cranial cavity.

Within a few minutes the animal was anesthetized and immobilized after a short time of excitation. About an hour later the animal recovered. If brought back into the basin it was in each instance found to behave normally the next morning and the following days. The experiment could be repeated on the same animal on the next day. It may be open to discussion whether the fading of the procaine effects is due to reabsorption and elimination, or to destruction of novocain by a procaine esterase. Up to the present time there has been no proof of the existence of a procaine esterase in frogs. In a previous paper, however, it was shown that the blood of turtles contains an amount of procaine esterase, the strength of which is between that of a rabbit and a cat (2).

## OBSERVATIONS ON FROGS ANESTHETIZED WITH INTRACRANIAL NOVOCAIN INJECTIONS

A few observations made on such animals in procaine narcosis may be mentioned. The first effect as a rule is a short excitation stage similar to that found in fish. At the beginning of the immobilization, leg reflexes could still be elicited. Pinching or pressing of the foot produced a flexor reflex of the homolateral leg. In deeper

narcosis sometimes also only a flexor reflex of the contralateral leg followed the stimulus. Even at a time when these reflexes could no longer be provoked in the frog suspended by both arms or lying on its back, the legs were always brought spontaneously to their normal position, if the frog was put on its belly. That happened spontaneously as well as in the way of a reflex after pinching a leg. At a time when the leg reflexes were still present, they were easily exhaustible (fatigue) and in some instance if tried two or three times in quick sequence they were no longer elicited. After a minute of rest they could again be produced.

The fact that the occurrence of these reflexes depends on the position of the animal could be shown in various ways. At a certain stage of anesthesia the leg reflexes could not be produced if the animal was lying on its back with its legs on the table, but could be produced easily if the animal was lying on its back as before but with the leg hanging over the edge of the table. Another reflex, easy to produce, also proved to be dependent upon the animal's position. If the animal were lying on its back it was possible by tapping its belly or its flanks or by lightly touching the ventral part of the upper thighs to provoke a croak reflex. This reflex could not be produced if the frog were lying on its belly.

In a former paper it was mentioned that fish narcotized by intracranial novocain injections showed at the beginning or the end of the narcosis a nystagmus (1) as previously seen in rabbits (3) during intravenous nembutal narcosis. In none of the frogs could anything similar to real nystagmus be seen. When the frogs recovered from narcosis, however, the following behavior could be observed. The eyes were rhythmically protruded and retracted and whenever the bulbi were protruded, the eyelids were opened wide, the nostrils closed and the flanks drawn in. This eye movement could either appear spontaneously or be provoked by touching an eye of the animal or its belly. It resulted sometimes in groups of such rhythmic contractions lasting a few minutes.

#### SUMMARY

A method is described for immobilizing and anesthetizing frogs by intracranial injection of approximately 0.5 cc. of a 3 to 5 per cent solution of novocain. The anesthesia which follows a short stage of excitation lasts about one hour. It is followed by complete recovery.

During the anesthesia the behavior of reflexes and the influence of the position of the animal on leg reflexes and croak reflexes were studied. During the time of recovery a kind of nystagmus equivalent appeared spontaneously, or it could be provoked as a reflex by touching the eyeball or the belly.

The kindness of Dr. Sergei Feitelberg in placing at my disposal material and equipment of the Physics Department of Mount Sinai Hospital is highly appreciated.

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# DESCENDING NERVE FIBERS SUBSERVING HEAT MAINTENANCE FUNCTIONS COURSING WITH THE CEREBROSPINAL TRACTS THROUGH THE PONTS<sup>1</sup>

ALLEN D. KELLER

*From the Department of Physiology, Baylor University College of Medicine*

HOUSTON, TEXAS

**D**URING a study concerned with determining whether any of the nerve fibers subserving heat maintenance functions decussate at the midbrain or pontine levels (1), we were led to suspect that some of these fibers descend from the hypothalamus in close association with the cerebrospinal tracts. The experiments described in this paper were designed to test this probability.

## METHODS

*Operative.* The dorsal aspect of the inferior colliculus was exposed by elevation and retraction of the occipital lobe. A small rigid probe was projected downward along the midline until the base of the skull was reached, then withdrawn slightly, after which it was pulled laterally through the soft tissue of the stem. In this way a hemisection of the stem was accomplished, except for sparing a small amount of tissue ventrally and medially. The same procedure was subsequently carried out on the opposite side. The end result was a complete transection of the dorsal aspects of the brain stem, leaving, from preparation to preparation, a varying amount of ventral tissue unsevered. It was calculated that in certain instances the only descending fibers left unsevered would be those in the pyramidal bundles (dog 76, fig. 1).

*Postoperative Management.* The animals were maintained in 28 to 30° C. incubators postoperatively until there was evidence (raised rectal temperature and shivering) that some heat maintenance functions were retained. They were then removed to hammocks at ordinary room temperature (24° to 26° C.). In the event no heat maintenance ability became evident, they were maintained continually in 28° to 30° C. incubators. On the morning after operation (20 hours postoperative), the animal's heat maintenance powers were assayed by placing it in an environment of 3° to 10° C. for a 6- or 8-hour period as previously described (2). After this test the animals were placed on a hammock in an environmental temperature of 24° to 26° C. and the rectal temperature followed for several hours. Some preparations were terminated on the second day after operation, while others were continued for several days. These latter were usually terminated by spontaneous death of the animals.

*Tissue: Method of Determining Extent of Lesion.* The animals which were terminated were done so under full sodium pentobarbital anesthesia by opening the thorax and cutting the superior vena cava. It was then perfused through the left heart with 0.9 per cent saline solution followed with 10 per cent neutral formalin. The cadaver was placed in a refrigerated box for several hours to allow for maximal hardening of the central nervous system. The brain was then removed, blocked and trimmed, and returned to formalin for a period of 24 hours. The extent of the lesion was determined a) by careful gross inspection after removal of the pia and b) by cutting through the unsevered tissue in a transverse direction or by cutting the brain stem sagittally along the midline.

In the animals which were found dead, the brain was removed carefully and placed in 10 per cent neutral formalin for 24 hours, at which time the pia was removed and the block containing the

Received for publication June 14, 1948.

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.

lesion was cut out and trimmed appropriately. This block was returned to 10 per cent formalin for an additional 24 or 48 hours.

### RESULTS

It was found that we could routinely section the greater part of the dorsal brain stem tissue at the level of the pons without materially affecting the animal's ability to combat a cold environment. There was some variable deviation from the normal in the heat maintenance powers in these animals, as shown by a mild drop in rectal temperature during the early part of the cold environment test and an overshooting of rectal temperature with a continuation of shivering beyond the termination of the test. They always, however, combatted successfully an environmental temperature of  $5^{\circ}$  to  $10^{\circ}\text{C}$ . for 6 to 8 hours, during which time the rectal

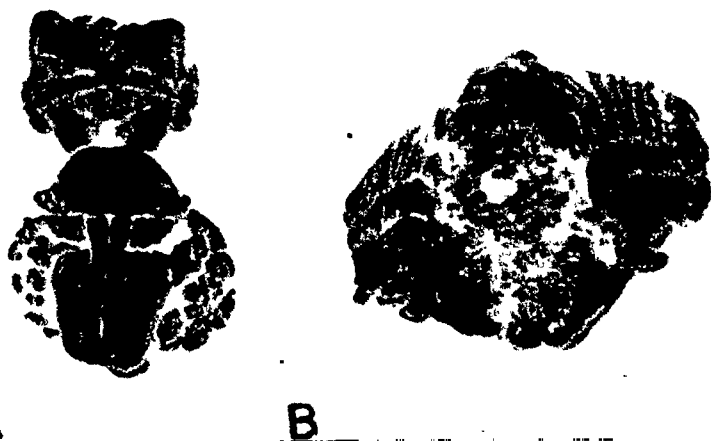


Fig. 1. A. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 76*, showing the level and extent of transection. B. Photograph of cross-section of caudal portion of brain stem after unsevered portion of pyramidal bundles was cut through with a razor blade. It is to be noted that the dorsal one third or one half of pyramidal bundles was severed by the operative procedure.

temperature fell at the most  $2^{\circ}\text{C}$ ., with prominent generalized shivering throughout the test.

The record for the 6-hour  $5^{\circ}\text{C}$ . test on *dog 85* is shown in figure 2C. It is to be noted that the deviation from the normal in this animal consisted of a) a drop in rectal temperature from  $39.4^{\circ}\text{C}$ . to  $38^{\circ}\text{C}$ ., which was regained during the test, b) the abrupt rise of the rectal temperature to  $41^{\circ}\text{C}$ . on cessation of the test, and c) a continuation of generalized shivering for several hours after the cessation of the test. It is to be noted from the photograph in figure 2A that the lesion cut through the lateral aspects of the pons, but that the entire medial portion of the pons remained undisturbed. The photograph in figure 2B shows the amount of the ventral tissue of the stem which remained unsevered. This can be seen to be the tissue of the pons proper.

It was also found, on the basis of several successful experiments, that we could routinely cut all the tissue of the brain stem except for the pyramidal bundles as they become exteriorized at the caudal extent of the pons, without eliminating all heat maintenance powers. In these instances there was a greater deficit during the test

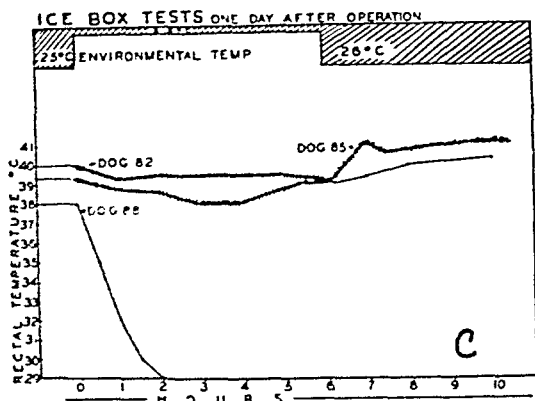
**A**



**B**



Fig. 2. *A*. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 85*, showing level and lateral extent of transection. It is to be noted that the lesion was symmetrical on both sides and involved only the lateral aspect of the pons ventrally. *B*. Photograph of medial aspect of right half of brain stem of *dog 85*, showing level and extent of transection. It is to be noted that a slight amount of the tissue immediately dorsal to the pons remained unsevered. *C*. Graph of cold environmental temperature tests on *dogs 82, 85 and 88*. See text for further details of these animals.



**A**



**B**

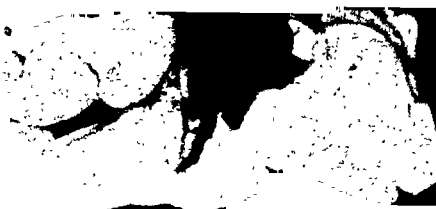
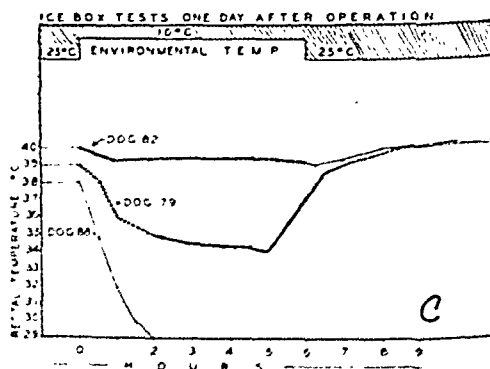


Fig. 3. *A*. PHOTOGRAPH OF VENTRAL SURFACE of brain of *dog 79*, showing level and extent of transection. Note that all of the tissue was transected at the caudal level of the pons, except for the pyramidal bundles. *B*. Photograph of medial aspect of left half of brain stem of *dog 79*, showing level and extent of transection. It is to be noted that all of the tissue at this level was completely transected except for the pyramidal bundles. For a short distance immediately dorsal to the pyramidal fibers, the lesion is not filled with blood clot and can be seen only by careful inspection as a thin tissue defect. *C*. Graph of cold environmental temperature tests on *dogs 79, 82 and 85*.



than in the former group, but considerable maintenance powers were always in evidence. These features were well shown in the experiment on *dog 79*, the results of which are illustrated in figure 3.

This dog's rectal temperature on the morning after operation was  $39^{\circ}\text{C}.$ , after being housed during the night on a hammock in a room at  $25^{\circ}\text{C}.$  When placed in a  $10^{\circ}\text{C}.$  environmental temperature, the animal immediately began atypical shivering (slight and periodic), and the rectal temperature fell precipitantly. After two hours, continuous vigorous shivering began, and the rate of fall in rectal temperature decreased accordingly.

In addition, it was found that considerable gross encroachment upon the dorsal aspects of the pyramidal bundles was compatible with the sparing of considerable heat maintenance powers. This was evident in *dog 76*, the extent of the transection being illustrated in figure 1. Although this dog died before a cold environmental test was run, it maintained a rectal temperature of  $38.5^{\circ}\text{C}.$  when housed on a hammock in a room at  $25^{\circ}\text{C}.$ , and under these conditions exhibited typical generalized shivering.

If the transection was complete, the animal exhibited no heat maintenance abilities (3). The response of a dog (*dog 88*), which had a complete transection except for a few strands of pyramidal fibers, illustrates this fact and is reproduced in both figures. This animal showed no evidence of any maintenance powers during the cold box test on the day after operation; however, it did possess remnantal powers as shown by a 'regulation interval' ranging from  $10^{\circ}\text{C}.$  the day after operation to  $13^{\circ}\text{C}.$  on the tenth postoperative day.

The responses of the above described animals to the cold environment test are contrasted in figures 2 and 3 with that of *dog 82*, which had the pons hemisected on the right side the day previous to the test. This dog exhibited a mild postoperative hyperthermia which frequently follows a major traumatic lesion at the level of the pons, but responded normally to the cold environment test.

#### DISCUSSION

Although the foregoing experiments were entirely acute in type, they clearly demonstrate that some thermogenic fibers, both shivering and nonshivering, descend as a component part of the pyramidal bundles at the level of the pons.

The experiments do not give any reliable indication as to the relative proportion of the over-all heat-maintenance fibers which descend through the pons with the cerebrospinal fibers. The fact that there was some deficit in heat maintenance powers in these animals does not necessarily indicate that the number of heat maintenance fibers coursing in the pyramidal bundles is inadequate to maintain normal maintenance functions, because some of the fibers may have been temporarily defunctioned by the lesion sequelae. This question can be answered only by chronic experiments.

To find thermogenic fibers coursing in the pyramidal bundles is of particular interest for two reasons. First, recent anatomical evidence has demonstrated that fibers other than those taking origin from the pyramidal or Betz cells course with the cerebrospinal tracts, many of which conform to the conventional anatomical

criteria for classifying them as autonomic fibers (4). Second, clinically vasomotor and related autonomic disturbances have long been observed on occasion to be associated with intracranial crises localized to or closely associated with the cerebrospinal tracts (5).

#### SUMMARY

The entire pons save for sparing the pyramidal bundles was transected in dogs without eliminating heat-maintenance powers.

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# EFFECT OF ALLOXAN, PANCREATECTOMY AND ADRENALECTOMY ON PLASMA AMINO NITROGEN IN THE DOG AS STUDIED BY MEANS OF HEMORRHAGE<sup>1</sup>

DANIEL L. KLINE<sup>2</sup>

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University*

NEW YORK CITY

ENDOCRINE influences in nitrogen metabolism have been investigated largely through studies of changes in N excretion and in the NPN level of the blood, produced as a result of hormone injection or removal of the gland concerned. In this manner it has been demonstrated that injection of the growth hormone (1) and of androgens (2) cause N retention, whereas injection of estrogens (3), thyroxine (4) and the adrenal steroids (5) increase N breakdown. From a study of the rate of accumulation of NPN in the blood of nephrectomized dogs, Mirsky (6) concluded that the administration of an anterior pituitary extract which contained the growth hormone induced N retention only when the pancreas was present. Lack of insulin was held responsible for this effect.

Recently, White and Dougherty (7) by analyses of various tissues have secured evidence which indicates that the cortical steroids cause mobilization of N from lymphoid tissue, and that the injection of thyroxine is followed by a removal of N from the carcass.

It is difficult to study the influence of hormones on N metabolism by means of plasma amino N changes because of the remarkable stability of the amino acid level of the blood. However, it has been shown in the rat, that following hemorrhage liver function is depressed (8), and the blood amino N level rises (9). A rise in the arterial amino N level and a significant femoral arterio-venous amino N difference, indicating the liberation of amino acids from the muscles of the leg, were demonstrated after hemorrhage in the dog (10).

In this paper are reported the arterial plasma amino N levels and the femoral A-V amino N differences in *a*) alloxan-treated, *b*) depancreatized and *c*) adrenalectomized dogs before and after a standard hemorrhage.

## PROCEDURE

Approximately 55 mgm/kgm. of alloxan were injected into each of a series of dogs. In a few instances additional, larger injections were made if the first dose proved inadequate to produce a sustained hyperglycemia. Only those animals in which the blood glucose remained above 185 mgm per cent were used. The experiments were performed at least three days after the last injection.

Received for publication June 7, 1948.

<sup>1</sup> A preliminary report of this work appeared in *Federation Proc.* 6: 143, 1947.

<sup>2</sup> Present address: Department of Physiological Chemistry, Yale University, New Haven, Conn.



The depancreatized animals were maintained with protamine Zn insulinate until the incisions had healed. No supplement was added to the ordinary diet of commercial 'Friskies'. Insulin was withdrawn two to five days before the experiments were performed.

The removal of the adrenal glands was carried out in two stages. Each gland was removed in one sharply delineated mass. After the second operation, the animals were maintained with commercial Doca and given 0.9 per cent saline to drink. The injections of Doca were stopped at least two days before the experiment. The 5 animals upon which successful experiments were carried out ate well and maintained their body weights after operation. Food was withheld for 14 to 24 hours prior to each experiment.

Comparable degrees of hemorrhage were produced without anesthesia by the method of Walcott (11). The animals were bled from, and 25 per cent of the bleeding volumes were immediately returned into, the right femoral artery. In the adrenalectomized dogs, 30 per cent of the bleeding volume was reinfused in an effort to extend their survival periods. None of the animals reported on in this paper survived the hemorrhage.

The procedure for obtaining simultaneous (within two minutes) blood samples from the left femoral artery and vein with minimum disturbance to the circulation has been described elsewhere (10). Blood samples were taken before bleeding and at hourly (half-hourly in the experiments on adrenalectomized animals) intervals until death occurred. Those taken within 20 minutes of death are designated as terminal.

Blood glucose was determined by the method of Somogyi (12) and plasma amino N was estimated by the manometric ninhydrin method (13). All data are presented in terms of mgm/100 cc. of plasma.

## RESULTS

*Arterial Plasma Amino N.* The average control arterial plasma amino N levels of the alloxan-injected, depancreatized and adrenalectomized dogs and the changes in these values after hemorrhage are shown in table 1. The average values which were found previously in a series of otherwise untreated, similarly bled dogs (10) are included for comparison. The survival periods have been divided into quarters, each approximating one hour, except for the adrenalectomized animals in which each quarter of the survival period averaged 20 minutes.

The arterial blood glucose levels of the alloxan-injected dogs averaged 258 mgm. per cent (range 187 to 375) and those of the depancreatized animals, 294 mgm. per cent (range 244 to 385).

1. *Before hemorrhage.* The average control arterial amino N value of 8 alloxan-injected dogs was  $4.41 \pm 0.45$  mgm per cent, and of 8 depancreatized dogs,  $7.27 \pm 0.73$  mgm. per cent, as compared with that of the control series which was  $3.65 \pm 0.10$  mgm. per cent. Statistically, there is a significant difference between each of the three series ( $p < 0.01$ ). Adrenalectomy in 5 dogs caused no change in the arterial level, the values averaging  $3.89 \pm 0.26$  mgm. per cent (table 1).

2. *After hemorrhage.* Both the control and the alloxan-injected animals showed

an increase in the arterial amino N levels after hemorrhage. The two series did not differ significantly from each other as to the magnitude of the increase which occurred. The depancreatized dogs, however, after a similar hemorrhage, showed no significant change in the concentration of arterial amino N. Since the depancreatized dogs had higher control values, the average arterial levels of the normal and alloxan-injected, bled animals approached that of the depancreatized dogs during the course of the hemorrhage. Statistically, the change from the control value of the depancreatized series differs significantly from that of the normal and alloxan series in each quarter of the survival period ( $p < 0.01$ ).

The adrenalectomized dogs proved so susceptible to stress that it was impossible to obtain survival times longer than one and one half to two hours despite the reinfusion of 30 per cent of their bleeding volumes, whereas only 25 per cent was returned to the dogs in the other series. However, it is evident (table 1) that despite an average survival time of only 80 minutes the arterial level rose in a manner compa-

TABLE 1. ARTERIAL PLASMA AMINO NITROGEN LEVELS (BEFORE AND AFTER HEMORRHAGE)

EXPERIMENT	PLASMA AMINO NITROGEN IN MG. PER 100 CC.						
	No. of Dogs	Control	Survival Time				
			No. of Dogs	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	Terminal
Normal . . . . .	35	$3.65 \pm 0.10^1$	16	$4.76 \pm 0.15$	$5.16 \pm 0.20$	$5.84 \pm 0.20$	$7.20 \pm 0.32$
Alloxan <sup>2</sup> . . . . .	8	$4.41 \pm 0.44$	8	$5.55 \pm 0.49$	$5.49 \pm 0.55$	$6.05 \pm 0.66$	$8.53 \pm 0.71$
Depancr. <sup>3</sup> . . . . .	8	$7.27 \pm 0.77$	5	$6.69 \pm 0.46$	$6.54 \pm 0.52$	$6.60 \pm 0.56$	$7.66 \pm 0.94$
Adrenx. . . . .	5	$3.89 \pm 0.26$	5	$4.86 \pm 0.25$	$5.54 \pm 0.21$	$6.17 \pm 0.25$	$6.92 \pm 0.37$

<sup>1</sup> Mean  $\pm$  standard error of mean  
<sup>2</sup> Average blood glucose 258 mgm. per cent (range 187-375).  
<sup>3</sup> Average blood glucose 294 mgm. per cent (range 244-385).

table to that observed in the control, bled dogs whose survival times averaged four hours.

*Femoral Arterio-venous Plasma Amino N Differences.* The femoral A-V amino N differences of the alloxan-treated, depancreatized and adrenalectomized animals before and after hemorrhage are shown in table 2. In all instances the venous level was higher, that is, amino N was added to the circulating blood as it passed through the leg.

1. *Before hemorrhage.* The A-V amino N differences averaged  $0.32 \pm 0.19$  mgm. per cent for the adrenalectomized,  $0.52 \pm 0.17$  mgm. per cent for the alloxan-injected and  $0.82 \pm 0.43$  mgm. per cent in the depancreatized dogs as compared with  $0.18 \pm 0.04$  mgm. per cent in the normal series (table 2). The difference between the normal and the alloxan series ( $p = 0.03$ ) and between the former and the depancreatized series ( $p = 0.02$ ) is of borderline significance. One of the animals injected with alloxan and two of the pancreatectomized dogs, however, showed a release of amino N into the blood far greater than was ever observed in the control animals. One of the adrenalectomized dogs had a negative A-V difference, that is, the concentration of amino N was less in the blood leaving than in that which entered the leg.

2. *After hemorrhage.* As a result of the type of hemorrhage used in these experiments, the blood flow through the leg in control and diabetic animals decreased to approximately 20 per cent of the pre-hemorrhage value (14). The femoral A-V differences tend to be magnified by the smaller quantity of blood flowing through the legs. After hemorrhage the average A-V difference of the alloxan-injected dogs was approximately one and one half, and that of the depancreatized animals was five times as great as the average value found in the control, bled series (table 2). Statistically, the post-hemorrhage A-V differences of the three series are significantly different from each other ( $p < 0.01$ ).

The adrenalectomized dogs showed no rise in their average A-V differences after hemorrhage, and, indeed, one animal had a negative control A-V difference which became increasingly more negative throughout the survival period.

TABLE 2. FEMORAL ARTERIO-VEIN AMINO NITROGEN DIFFERENCES (BEFORE AND AFTER HEMORRHAGE)<sup>1</sup>

EXPERIMENT	A-V AMINO NITROGEN DIFFERENCES IN MGM. PER 100 CC.						
	No. of Dogs	Control	Survival Time				
			No. of Dogs	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{2}{3}$	Terminal
Normal.....	16	$0.18 \pm 0.04$	10	$0.51 \pm 0.08$	$0.71 \pm 0.12$	$0.66 \pm 0.14$	$0.45 \pm 0.10$
Alloxan....	8	$0.52 \pm 0.17$	8	$0.97 \pm 0.14$	$1.24 \pm 0.20$	$1.40 \pm 0.26$	$0.79 \pm 0.44$
Depancreat.	5	$0.82 \pm 0.43$	5	$2.73 \pm 0.72$	$3.25 \pm 0.69$	$3.01 \pm 0.55$	$3.14 \pm 0.81$
Adrenx....	4	$0.32 \pm 0.19$	4	$0.31 \pm 0.25$	$0.21 \pm 0.49$	$0.20 \pm 0.47$	$0.11 \pm 0.33$

<sup>1</sup> In all instances the venous levels were higher. See footnotes to table 1.

The blood volumes, bleeding volumes and survival times of the control, alloxan-injected and depancreatized animals were almost identical. In addition, measurements of the blood flow through the femoral vein before and after hemorrhage (14), show remarkable agreement between the three series of animals in the vascular response to the hemorrhage. From these measurements, it would appear that the changes in plasma amino N levels observed may be ascribed to metabolic rather than purely vascular differences between the types of experimental animals used.

#### DISCUSSION

A significant elevation in the arterial amino N level was found in alloxan-injected and depancreatized animals within one week following the production of hyperglycemia (table 1). The addition of amino N to the blood as it passed through the leg was also greater in diabetic animals than in control dogs (table 2).

The significance of alterations in the plasma amino N level is not known although values above normal are usually associated with liver damage. An elevation in the blood amino N level has been described after experimental pancreatectomy (15), and in human diabetics (16, 17). The latter authors reported that insulin lowered the blood amino N level of their patients. A decrease in the blood amino N level after the injection of insulin into normal animals has also been described (18).

Differences in amino N metabolism between alloxan-injected and depancreatized dogs, in which the extent of hyperglycemia was approximately the same, are indicated by the data obtained. The arterial amino N level of the depancreatized dogs was significantly higher than that of the animals injected with alloxan. A further difference appears in the response to hemorrhage. Whereas the arterial level of the dogs injected with alloxan increased in a manner closely resembling the response obtained in control, bled dogs, hemorrhage produced no significant alteration in the arterial amino N values in animals from which the pancreas had been removed. The changes in the concentration of amino N as the blood passed through the leg likewise reveal significant differences between the two series, the values being greater in the depancreatized animals. Hemorrhage did not alter this relationship; the A-V difference in each series increased proportionately, and the liberation of amino N from the muscles was greater in depancreatized than in alloxan-injected animals.

The failure of a post-hemorrhagic rise in the plasma amino N level to occur in depancreatized dogs cannot be ascribed to increased excretion in the urine, since, following the hemorrhage, the animals were anuric. In the depancreatized dog subjected to the stress of hemorrhage, as compared with intact or alloxan-treated animals under the same conditions, therefore, much larger amounts of amino N were removed from the blood stream by the liver.

The elevated arterial amino N levels of the diabetic animals may be caused by a disturbance of the equilibrium between amino acid production and removal, resulting in a shift of the equilibrium toward a higher plasma amino N level. This 'higher setting of the thermostat', however, is not a reflection of an impaired ability of the liver to remove amino acids because in the depancreatized animals, in which peripheral production was the greatest, the arterial amino N level did not rise after hemorrhage.

A direct toxic effect of alloxan upon the liver may explain the differences in amino N metabolism observed between the alloxan-injected and the depancreatized animals, although those animals which were injected several times with increasing amounts of alloxan showed no difference in response from animals injected only once. It is also possible that in the depancreatized animal deprived of many of its proteolytic enzymes, the mobilization and utilization of endogenous nitrogen sources may proceed at an accelerated rate. Until this possibility is explored, the postulation of a second hormone in the pancreas would not appear to be necessary.

The adrenalectomized animals cannot be compared directly with the other series because of differences in the vascular response and the marked susceptibility of these animals to stress. However, the animals showed amino N changes the magnitude and direction of which are of interest. Thus, the average arterial amino N level of the adrenalectomized dogs did not differ from that of the untreated series either before hemorrhage or in any quarter of the survival period even though their total survival times averaged only 80 minutes as compared with 4 hours for the control, bled series (table 1). The average femoral A-V difference of the adrenalectomized animals, in contrast to the results obtained from control, bled dogs, showed no rise following hemorrhage. This observation lends support to the theory ad-

vanced by Long, Katzin and Fry (19) that the adrenalectomized animal is unable to mobilize its endogenous nitrogen at a normal rate.

Comparison of the rates of peripheral production of amino N after hemorrhage as measured by femoral A-V differences in depancreatized and adrenalectomized animals reveals no correlation between the output of amino N from the muscles and the arterial levels observed. For example, despite the fact that there was no increase in the liberation of amino acids after hemorrhage in adrenalectomized animals, the arterial level increased as much in 80 minutes as it had in 4 hours in normal, bled dogs. On the other hand, depancreatized dogs showed no increase in the arterial level after hemorrhage in spite of a large addition of amino acids from the legs. It would appear, therefore, that the plasma amino N level in these experiments was not dependent upon the rate of production but that regulation of the arterial level was determined by the rate of removal of amino acids from the circulation.

#### SUMMARY

Arterial plasma amino N levels and femoral arteriovenous amino N differences were determined in alloxan-injected, depancreatized and adrenalectomized dogs before and after a standard hemorrhage, and the values obtained were compared with those from a series of normal, bled animals.

The average control arterial level was elevated in alloxan-injected dogs, 4.41 mgm. per cent, and was much higher in depancreatized animals, 7.27 mgm. per cent, as compared with the control series, 3.65 mgm. per cent (table 1). Following hemorrhage the average arterial level rose in the alloxan-injected and normal animals but showed no change in the animals of the depancreatized series.

The average control femoral A-V amino N differences, which indicate the addition of amino acids to the blood from the muscles of the leg, were higher in alloxan-injected, and much greater in depancreatized, than in normal animals. After hemorrhage the A-V differences increased in each series, maintaining, however, the same proportionate relationship (table 2).

Significant differences between the alloxan-injected and the depancreatized dogs appeared in *a*) the arterial level, *b*) the response in the arterial level after hemorrhage and *c*) the A-V amino N difference.

The average control arterial level of the adrenalectomized dogs and the rise which occurred in each quarter of the survival period after hemorrhage did not differ from that of the animals of the normal series even though their survival times averaged only one-third that of the normal series. The average femoral A-V amino N differences in adrenalectomized dogs showed no increase after hemorrhage.

The author is indebted to Dr. Walter S. Root and Dr. Alfred E. Wilhelmi for their valuable assistance in the preparation of the manuscript.

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# ALLOXAN DIABETES IN SHEEP UNDER FASTING AND NON-FASTING CONDITIONS

ESTHER L. McCANDLESS<sup>1</sup>, BARBARA A. WOODWARD AND J. A. DYE

*From the Department of Physiology, Cornell University*

ITHACA, NEW YORK

INVESTIGATION of the diabetic state in the calf (1, 2) and goat (3) have indicated that the processes regulating carbohydrate metabolism in ruminants differ in certain respects from those of carnivores, from which much of the knowledge of carbohydrate metabolism has been derived. As von Mering and Minkowski (4) showed in 1889, pancreatectomy in the dog results in a syndrome similar to severe diabetes mellitus in man. Subsequent work has shown that hyperglycemia, ketonemia, and marked increases in glucose, ketone, and nitrogen excretion in the urine accompany the clinical signs of polydipsia, polyuria and polyphagia in this group of animals.

In the depancreatized calf, however, Cook and Dye (1) reported a very mild form of diabetes. Hyperglycemia was present when the calf was fed, but hypoglycemia developed when feed was withheld. Greeley (3) found that the depancreatized goat can maintain a healthy state, even without the administration of insulin.

The discovery of Dunn *et al.* (5), that intravenous administration of alloxan monohydrate, a derivative of uric acid, causes necrosis of the insulin-producing cells of the pancreatic islets in rabbits, has opened a new era in research on intermediary metabolism. McCandless and Dye (2) reported low glucose tolerance and glycosuria, but normal blood glucose levels, in a calf treated with alloxan. Alloxan diabetes in goats was found to be moderately severe by Saviano (6). Jarrett (7) observed severe diabetes in sheep treated with alloxan, and a high degree of susceptibility to the toxic effects of the drug. In a preliminary paper from this laboratory (8), studies of 2 alloxan diabetic ewes were reported. The present paper is a continuation of that work.

## METHODS

Alloxan diabetes was produced in 8 grade sheep by the intravenous administration of alloxan monohydrate (Eastman), in doses of 125, 100, and 75 mg. per kgm. in *sheep* 1, 2, and 3 through 8, respectively. The drug was dissolved, immediately before use, as a 5 per cent solution in sterile distilled water. It was then injected rapidly into the jugular vein. Blood samples were taken for glucose determination immediately preceding the injection, and at selected intervals during the first 24 hours. On subsequent days, blood glucose was determined at 8 or 9 a.m. and an aliquot of the 24-hour urine was analyzed for glucose, ketones and nitrogen. Nitrogen determinations could not be carried out on some samples because of fecal contamination. The blood was analyzed for ketones several times in the experimental period of each animal.

<sup>1</sup>Received for publication May 24, 1948.

<sup>2</sup>Recipient of fellowships from the Gans Fund of Bethany College, Bethany, W. Va., 1946 and 1947.

After preliminary studies of blood and urine chemistry, all food was withdrawn and the sheep were studied under fasting conditions. It should be pointed out that the ruminant continues to absorb nutrients for several days after the initiation of the fast, as a consequence of the large amount of material in the rumen. Experimental observations in this laboratory indicate that postabsorptive conditions are not attained until the 3rd or 4th day of the fast.

The survival period of each animal was determined. Immediately after death, pieces of pancreas, liver and kidney were removed, fixed both in Bouin's and in Zenker's solutions, and imbedded in paraffin. The tissue sections were stained with hematoxylin and eosin.

Blood and urine glucose was determined by the Somogyi-Shaffer-Hartmann method (9); urine glucose was corrected for non-fermentable reducing substances by the use of a yeast blank. Muscle and liver glycogen was determined by the method of Good *et al.* (10), and similarly corrected for non-fermentable reducing substances. Ketones were determined by the Ravin modification of Behre-Benedict method (11), and nitrogen by the macro-Kjeldahl method (12).

#### RESULTS

*Initial Response to Alloxan Injection.* A triphasic glycemic response, namely, initial hyperglycemia, secondary hypoglycemia and then permanent diabetic hyperglycemia, was observed in all cases except *sheep 5* (table 1). Following the administration of alloxan, the blood glucose level rose steadily, reaching its peak of 92 to 217 mgm. per 100 ml. within 4 to 8 hours. A maximal hypoglycemia of 14 to 31 mgm. per 100 ml. was observed at the 9th to the 14th hours. Despite the low values of blood glucose, no symptoms of hypoglycemia were present. This phase was missed in *sheep 5*, in which hyperglycemia was present each time the blood glucose was determined, namely, at 4, 9, 11, and 13 hours postinjection.

Twenty-four hours after the injection, diabetic hyperglycemia had been established in all but one of the sheep. In *sheep 1*, which showed a prolonged hypoglycemia, blood glucose did not ascend to diabetic levels until the 38th hour.

Toxic effects of alloxan were observed in two animals. *Sheep 1* became weak 13 hours after receiving 125 mgm. of alloxan per kgm. Anuria was present from the 11th hour, and despite the administration of large amounts of saline and diuretics, 8t continued until death at 85 hours. As a consequence of renal failure, retention of glucose, nitrogen, and ketones was severe; 72 hours after the injection of alloxan, the blood glucose level was 665 mgm. per 100 ml., blood nitrogen 167 mgm. per 100 ml. and blood ketones 36 mgm. per 100 ml. Histological studies showed both hepatic and renal tubular damage.

In *sheep 5*, which received 75 mgm. of alloxan per kgm., renal failure did not become apparent immediately; the blood non-protein nitrogen had been normal on the 8th day. Two days later, the blood glucose level rose from 233 to 341 mgm. per 100 ml., although the animal was fasting. On the 11th day, the hyperglycemia had reached 852 mgm. per 100 ml. Administration of 180 units of crystalline zinc insulin (Lilly) in the next 24 hours lowered the blood glucose to 75 mgm. per 100 ml. How-



ever, renal damage, evidenced by anuria present from the 10th day, caused death 12 days after alloxan administration. Autopsy and histological studies showed glomerular and tubular damage in the kidney and fatty changes in the liver.

*Alloxan Diabetic State.* a) *Metabolic studies.* It can be seen from table 2 that the blood glucose levels of diabetic sheep are three to four times higher than those of normal sheep, in which the range is 35 to 65 mgm. per 100 ml. That the

TABLE 1. INITIAL GLYCEMIC RESPONSE TO ALLOXAN INJECTION, MGM. PER CENT

HOURS POST- INJECTION	SHEEP NO.							
	1	2	3	4	5	6	7	8
0	45	56	38	40	54	63	40	56
1/2	79		56	35				
1	88	118	56	45				
2	118	167	68	56		154	58	84
3	143	191	84	79				
4	165	200	91	107	165	182	114	217
5	167	189	92	121				
6	121	169				165	140	173
7	77	140	42	99				
8	26					140	134	112
9	22	77	14	54	184			
11	14		22	29	160	45	72	25
12	24	26						
13	22		19	26	165	31		
14	19						26	22
15		33	26	35		47		
16	19							
18		70						
19	17							
21		129						
22	24							
24		167	169	191	189	189	101	204
25	35							
27	47	184	193	209			158	255
30	38	191						
31			176					
33	68	211						211
38	213							

hyperglycemia is in large part alimentary is apparent from the comparison of values before and after fasting periods (table 3). In two cases, *sheep* 3 and 7, normal values of blood glucose, urine glucose, and urine ketones were obtained after fasts of 6 and 5 days, respectively. When the fast was prolonged in *sheep* 3, the blood glucose rose again to hyperglycemic levels.

In *sheep* 6, the first series of insulin treatments (protamine zinc insulin, Lilly) led to an increase in blood glucose, from 187 to 281 mg. per 100 ml., as a result of a great increase in food intake when on insulin therapy; this 5 months old ewe, with a body weight of 17 kgm., consumed approximately twice the adult intake of hay,

grain and commercial calf food in this period. Although the insulin dosage, 16 to 20 units daily, was inadequate at this time, the same dose proved to be too high at a later date when food intake was lower.

TABLE 2. VALUES OF BLOOD GLUCOSE AND KETONES, URINE GLUCOSE, KETONES, AND NITROGEN IN THE FED ALLOXAN DIABETIC SHEEP; AVERAGE VALUES AND RANGE

SHEEP NO.	POST-INJECTION	INITIAL WEIGHT	BLOOD GLUCOSE	BLOOD KETONES	URINE GLUCOSE <sup>1</sup>	URINE KETONES <sup>1</sup>	URINE NITROGEN
	days	kgm.	mgm. %	mgm. %	g/kgm/day	mgm/kgm/day	mgm/kgm/day
2	1-9	26.4	218 (202-246)	14	3.62 (0.31-5.61)		
3	1-11	47.3	171 (162-191)	24	2.39 (0.001-4.07)	131 (1.1-343)	507 (215-672)
4	1-10	36.8	175 (156-191)	47	1.64 (0.12-3.29)	129 (5.2-259)	418 (364-512)
5	1-3	44.1	187 (178-195)	51	1.75 (1.41-1.94)	160 (8.1-303)	400 (364-437)
6	1-12	17.0	173 (154-195)	41	1.98 (0.30-2.71)	238 (28.4-480)	415 (316-535)
7	1-9	17.7	148 (140-158)	11	1.00 (0.03-1.45)	4.1 (1.0-10.6)	362 (310-416)
7	10-20		146 (141-156)		1.38 (1.14-1.77)	24.5 (15.4-38.1)	341 (313-361)
8	1-7	27.7	190 (169-222)	12	1.65 (0.15-2.13)	102 (6.1-166)	316 (88-495)

<sup>1</sup> Low values on day 1.

TABLE 3. EFFECT OF FASTING IN ALLOXAN DIABETIC SHEEP

SHEEP NO.	FASTED	BLOOD GLUCOSE		BLOOD KETONES		URINE GLUCOSE		URINE KETONES		URINE NITROGEN	
		initial	terminal	initial	terminal	initial	terminal	initial	terminal	initial	terminal
	days	mgm. %		mgm. %		g/kgm/day		mgm/kgm/day		mgm/kgm/day	
2	6	206	173			2.79	0.15				
3	6	165	54			2.51	0.03	146	1.4	467	29
3	5	173	103			1.50	0.01	35	22	203	—
4	5	154	136			1.32	0.21	134	22	401	174
6	8	167	147			1.79	0.11	140	42	430	300
6	4	281	178	41	42	2.71	1.07	75	42	476	—
7	5	156	33			1.60	0.002	20	4	354	104
8	5	222	167	12	21	2.13	0.67	160	73	345	259

Glycosuria was severe in all sheep. The highest values were observed in *sheep* 2, which excreted over 5 grams of glucose per kgm. per day on the 2nd, 3rd, and 4th days after alloxan administration. Her blood glucose level never fell below 200 mgm. per 100 ml. during the period of study, except when the animal was fasting or receiving insulin. Glucose excretion was lowest in *sheep* 7, in which the blood glucose never rose above 158 mgm. per 100 ml. Nevertheless, it is believed that alloxan

damage to the pancreatic islets was complete, because a second injection of alloxan, on the 9th day, did not produce the typical initial glycemic fluctuations nor did it increase the degree of permanent diabetic hyperglycemia.

Fasting caused a rapid diminution of glycosuria in each of the alloxan diabetic animals. Normal values were obtained in all but 2 cases, the second fast of *sheep 6* and the fast of *sheep 8*. A marked drop in glucose excretion noted in the other sheep permitted the determination of the renal threshold at 140, 150, 150, 170, and 200 mgm. per 100 ml. of blood, in *sheep 4, 3, 7, 6, and 2*, respectively. When the blood glucose fell below these levels, less than 0.5 grams of glucose per kgm. per day was excreted in the urine. No correlation was apparent between the renal threshold and the ability of these animals to maintain the blood glucose level on fasting.

Glucose tolerance curves were studied in both normal and diabetic sheep, following the intravenous injection of 1 gram of glucose per kgm. body weight (fig. 1). Compared with the dog and calf, the normal non-fasting sheep had a low glucose tolerance, similar to that obtained in an alloxan diabetic dog studied in this laboratory. The blood glucose level of the sheep did not return to the preinjection level until 4 to 6 hours after glucose administration, as compared with 1 to 2 hours in the normal dog and calf. Diabetes did not change the slope of the tolerance curve in sheep; the return to the preinjection level occurred 5 to 6 hours postinjection in most instances, although occasionally blood glucose remained slightly elevated throughout the remainder of the day. It must be remembered, however, that the initial level of blood glucose in the diabetic animal was three to four times higher than normal.

It is apparent from figure 2 that fasting caused a marked decrease in glucose tolerance in both normal and diabetic sheep. The high postinjection blood glucose levels did not return to normal during the course of the day in these animals.

Nitrogen excretion was increased in the diabetic animals. Control studies established the average normal non-fasting value at 220 mgm. of ammonia nitrogen per kgm. per day. After the administration of alloxan this rose to 396 mg. per kgm. per day. Although this approximately twofold increase is significant, it is much less than that reported in depancreatized dogs. Fasting decreased the nitrogen excretion in all sheep, but to the greatest extent in *sheep 3* and *7*, both of which failed to maintain hyperglycemia under these conditions. If urinary nitrogen excretion can be used as an index of the degree of gluconeogenesis from protein in the sheep, it seems doubtful that protein is of great importance as a source of carbohydrate, in these 2 animals in particular.

Disturbances in fat metabolism were observed in all animals which survived the first 24 hours. Accompanying the loss of body weight, ketonemia rose in these sheep from the normal level of 3 to 5 mgm. per 100 ml., to 11 to 51 mgm. per 100 ml. Ketosis was slow to develop in *sheep 7* and never became severe. The blood ketone level had decreased from 24 to 6 mgm. per 100 ml. in *sheep 3* by the 45th day, from 41 to 16 mgm. per 100 ml. in *sheep 6* by the 78th day, and from 11 to 9 mgm. per 100 ml. in *sheep 7* by the 94th day of alloxan diabetes. Blood ketone determinations were limited in number for any given animal because of the large blood sample required.

Ketonuria increased steadily from the normal value of 1 to 5 mgm. per kgm. per day in *sheep* 3, 4, 5, 6, and 8, reaching a maximum of 166 to 480 mgm. per kgm. per day about a week after the production of diabetes. *Sheep* 3 excreted a total of 16 grams of ketone bodies on the 8th day. Although the excretion values dropped after the peak had been reached, normal levels were not attained in these animals, except in *sheep* 3 and 7 under conditions of fasting. Although the excretion of ketones was greatly decreased in the absence of a dietary source, this was apparently a renal phenomenon; the blood ketone level remained elevated in *sheep* 6 and was doubled in *sheep* 8 after fasts of 7 and 5 days, respectively. This suggests that the renal threshold for ketone bodies may be raised during fasting.

b) *General condition and survival* (table 4). Of the 8 sheep studied, 5 animals presented a metabolic picture of uncomplicated alloxan diabetes. The data for *sheep* 1 and 5, with renal impairment, and *sheep* 4, which was in poor condition when

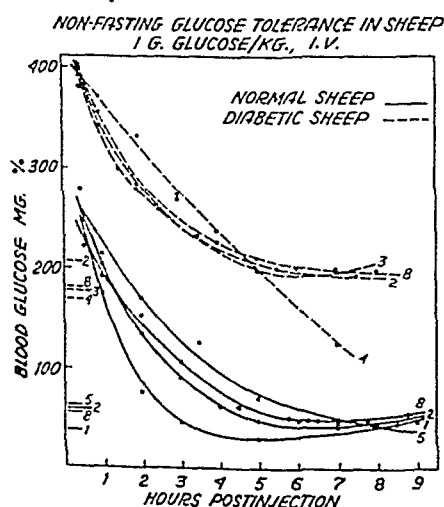


FIG. 1

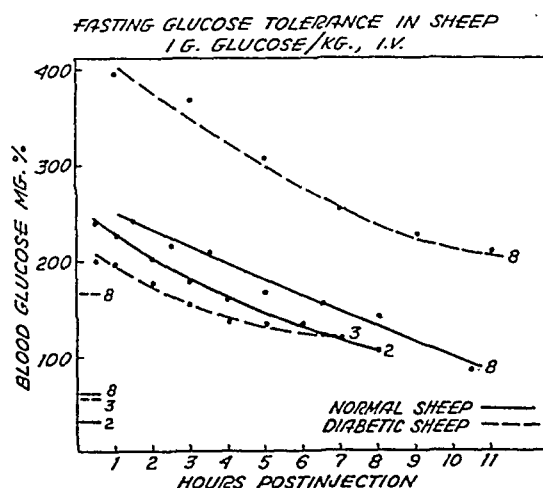


FIG. 2

the experiment was started, are for these reasons excluded from this section. The general condition of the other sheep remained good for 1 to 5 months, despite the initial weight loss.

In the first 2 weeks of alloxan diabetes, *sheep* 2, 3, 6, and 7 lost 17, 10, 11, and 15 per cent, respectively, of their body weights. At this time polyphagia became pronounced, and increased food intake compensated to some extent for the loss of glucose through the kidneys. Weight loss was excessive during periods of fasting. This is due, in part at least, to loss of rumen content.

*Sheep* 2 was in good condition for about 45 days, at which time it developed weakness and failed rapidly. Insulin therapy was begun on the 50th day; 40 to 48 units of protamine zinc insulin (Lilly) were administered daily. The animal went into severe hypoglycemic coma on the 60th day, with a blood glucose level of 8 mgm. per 100 ml. Although glucose was administered intravenously several times during the next 24 hours, the condition did not improve; the animal was killed on the 61st day. The terminal glycemia was 10 mgm. per 100 ml.

*Sheep* 3 remained in excellent condition for 150 days, maintaining body weight

by increased food intake during this period. An unfortunate mishap, not connected with the diabetes, resulted in death on the 157th day.

An 8-day fast in *sheep 6* caused a weight loss of 32 per cent, based on the initial weight. Insulin therapy was started on the 30th day of diabetes. Although 16 to 20 units of protamine zinc insulin (Lilly) were injected daily into this 17 kgm. lamb, the blood glucose level rose as a result of a great increase in food intake. Nevertheless, the animal regained weight and strength, and insulin was discontinued 16 days after its initiation. Progressive weakness was observed again from the 66th to the 71st day of diabetes, at which time insulin therapy was resumed. Two days later the lamb was comatose, shaking and salivating excessively. The blood glucose level was 22 mgm. per 100 ml. Intravenous administration of glucose brought the animal back to consciousness, but the weakness continued. The sheep was killed 78 days after the injection of alloxan, 6 days after the last insulin injection. The terminal blood glucose and ketones were 195 mgm. and 16 mgm. per 100 ml., re-

TABLE 4. SURVIVAL OF ALLOXAN DIABETIC SHEEP

SHEEP NO.	BREED	SURVIVAL	INSULIN THERAPY	CAUSE OF DEATH
1	Hampshire	85 hr.	None	Renal and hepatic damage
2	Hampshire	60 d.	days 50-58: 40-48 U protamine	Insulin hypoglycemia, following severe diabetic symptoms
3	Dorset	157 d.	None	Accidental
4	Hampshire	24 d.	None	Poor appetite and condition at start of experiment
5	Hampshire	12 d.	day 11: 120 U crystalline	Renal damage
6	Hampshire	78 d.	days 30-46, 71, 72: 16-20 U protamine	Severe effects of diabetes; killed
7	Shropshire	94 d.	none	Progressive weakness; killed
8	Hampshire	29 d.	day 21: 20 U protamine	Progressive weakness; killed

spectively. Although muscle glycogen was low normal, 0.38 per cent, liver glycogen was almost nonexistent, 0.04 per cent.

*Sheep 7* was killed on the 94th day of alloxan diabetes. The animal's condition had remained good for over 2 months, but failed in the last few weeks and the sheep stood only when feeding during the last 2 or 3 days. Although the weight loss in 3 months of alloxan diabetes appeared to be slight, 1.3 kgm., it should be remembered that *sheep 7* was a growing lamb which should have been gaining weight in this period. The terminal blood glucose was 235 mgm. per 100 ml., ketones 9 mgm. per 100 ml. In this animal, muscle glycogen was normal, 0.43 per cent, but the liver was free of glycogen.

*Sheep 8* showed progressive weakness from the 18th day, and was killed on the 29th day, 8 days after a single dose of 20 units of protamine zinc insulin (Lilly). The terminal blood glucose was 96 mgm. per 100 ml., terminal ketonemia 21 mgm. per 100 ml.; muscle glycogen was 0.46 per cent, liver glycogen 0.08 per cent. In one month of alloxan diabetes, this animal had lost 27 per cent of its body weight.

c) *Histological studies* (table 5). The renal damage which followed the injection of 125 and 75 mgm. of alloxan per kgm. in *sheep 1* and *5*, respectively, has been mentioned earlier. A large amount of ascitic fluid was removed from the peritoneal cavity of the former at autopsy, and other signs of renal failure were observed. Histological examination confirmed the diagnosis of coagulation necrosis of the convoluted tubules; many tubular casts were present. In *sheep 5*, the toxic effects of alloxan were apparent in both tubules and glomeruli; the latter were non-functional, compact masses of cells. *Sheep 3*, which died as a result of an accident, showed acute renal changes which were believed to result from the accident rather than from the diabetes. Renal pathology observed in three other animals consisted

TABLE 5. HISTOLOGICAL STUDIES OF ALLOXAN DIABETIC SHEEP

SHEEP NO.	RENAL HISTOLOGY	HEPATIC HISTOLOGY	PANCREATIC HISTOLOGY
1	Tubular necrosis	Infiltration and proliferation of duct cells	No evident pathology
2	No evident pathology	Vacuolation of cells	Decreased number of islets
3	Acute hemorrhagic areas; tubular necrosis	Poorly preserved	Poorly preserved
4	No evident pathology	Vacuolation of cells; prominent bile ducts	Decreased number of islets
5	Vacuolation of tubular cells; massive proliferation of glomerular cells, glomeruli compact	Vacuolation of cells; prominent bile ducts	Decreased number of islets
6	Proliferation of glomerular cells	Connective tissue stimulation in region of portal vessels; some hepatic cells clear	No islets identified
7	Proliferation of glomerular cells	Vacuolation of cells; connective tissue stimulation in perivascular regions	One islet identified in section; possible remnants of other islets
8	Vacuolation of tubular cells	Vacuolation of cells; connective tissue stimulation in perivascular regions	Decreased number of islets; islets small

of proliferation of cells of the glomeruli in two and vacuolation of the tubular cells in one.

From table 5, it is apparent that hepatic changes were marked in all animals. In *sheep 1*, these were probably the result of the toxicity of alloxan. In those sheep which survived for longer periods, fatty infiltration of the liver was evident.

No islets could be identified in the pancreatic tissue of *sheep 6*; in the other animals there appeared to be a decrease in the number present. No pathological alterations were apparent in these with the routine hematoxylin and eosin staining technique. The islet cell types could not be differentiated in normal sheep pancreas even when stained according to the Gomori chrome hematoxylin technique (13). Pancreatic islet cells in the sheep are mainly nongranular, and the alpha and beta cells are stained very faintly (14).



## DISCUSSION

The triphasic glycemic response to alloxan administration, observed in the sheep of this series, is typical of that presented by most other species studied. Although agreement is lacking in the explanations offered for the initial hyperglycemia and the secondary hypoglycemia, (15-18), all experimental results point to one cause for the third phase, the diabetic hyperglycemia. Assays of pancreatic tissue from alloxan diabetic animals have shown this to be the result of insulin deficiency, which is produced by the destructive action of alloxan on the beta cells of the pancreatic islets (15, 19). Studies of the alloxan diabetic state, for this reason, should provide a key to the action or actions of insulin in the normal animal.

Jarrett (7) observed severe but uncomplicated diabetes in 2 of 12 ewes treated with 88 to 200 mgm. of alloxan per kgm. One animal failed to develop diabetes; 2 were killed in the first 24 hours for histological studies, and 7 had serious hepatic and renal complications. Hyperglycemia, glycosuria and ketonuria were marked in both of the survivors, and large doses of insulin were required for control of the diabetes. The protocols of these animals are similar to those of our series.

In our sheep, intravenous administration of 75 mgm. of alloxan per kgm. resulted in uncomplicated diabetes in five cases, and in diabetes with renal involvement in one, *sheep 5*. In this instance, the fault appeared to be in the alloxan preparation, since it proved fatal to each of 5 rats to which it was administered in the usual manner and quantity.

*Sheep 1*, which received 125 mgm. of alloxan per kgm., died in 85 hours with coagulation necrosis of the renal convoluted tubules. No abnormality traceable to the toxic action of alloxan itself was found in the histological study of hepatic and renal tissue of *sheep 2*, 2 months after the injection of 100 mgm. of alloxan per kgm. It is apparent that 75 mgm. of alloxan per kgm. is the diabetogenic dose for sheep, and that doses greater than this may result in severe damage of the liver and kidneys.

After the initial period of readjustment to the metabolic abnormality, the glucose balance of the diabetic animals remains relatively constant, although this constancy is achieved through a turnover process in which the materials themselves are always changing. It is interesting to study the amount of carbohydrate present in the diabetic sheep in comparison with that in the normal. Muscle glycogen did not change significantly after the development of the diabetic state. The increase in blood glucose, and therefore an equivalent increase in tissue fluid glucose, is large in degree (122 mgm. per 100 ml.) but small in quantity (about 0.4 gram per kgm. body wt.). This increase does not balance the loss of liver glycogen (1.2 grams per kgm. body wt.), probably the most significant change in the glucose stores of diabetic sheep. From these results, it is apparent that the mechanism for net storage of glycogen in the liver is deficient, or that glucose mobilization from this organ is excessive even when the concentration of blood glucose without the aid of insulin maintains the muscle glycogen stores at approximately normal levels.

Despite the absence of liver glycogen, the total carbohydrate turnover of the diabetic sheep is higher than that of the normal sheep, when the amount of glucose excreted in the urine is taken into account. The source of the extra glucose in the

blood and urine of the diabetic carnivore is generally conceded to be the result of two processes: *a*) overproduction of glucose through hepatic gluconeogenesis and *b*) relative underutilization of glucose by the extrahepatic tissues.

Gluconeogenesis from body protein appears to be secondary as a source of blood glucose in diabetic sheep. The urinary nitrogen excretion was not quite doubled in these animals, and was less during fasting states, indicating a low degree of protein catabolism in both instances. The fed diabetic sheep excreted nitrogen equivalent to 2.4 grams of protein per kgm. per day, as compared to 1.5 grams per kgm. per day in the fed normal sheep. The fasted diabetic sheep excreted nitrogen equivalent to 0.2 to 1.8 grams of protein per kgm. per day. Little more than a gram of glucose per kgm. per day could arise, therefore, from protein catabolism in either fed or fasted conditions.

According to Barcroft *et al.* (20), the lower fatty acids arising from bacterial action on cellulose in the rumen are of great importance in the energy balance of ruminants. When absorbed, these may assume a major rôle in carbohydrate synthesis in this group of animals. Lorber and co-workers (21) have reported the appearance of labeled isotopic carbon atoms in liver glycogen produced *in vivo* from acetic acid. Other literature on gluconeogenesis from fat has been reviewed by Soskin and Levine (22). The presence of carbohydrate-fermenting bacteria in the rumen makes it doubtful that much, if any, glucose is absorbed as such into the portal circulation. Under these conditions, exogenous glucose is eliminated as the main source of blood glucose; gluconeogenesis is necessary for the maintenance of the blood glucose level in normal, non-fasting ruminants.

The type of glucose tolerance curve obtained in normal non-fasting sheep is similar to that of diabetic carnivores. In the latter, the low tolerance is the result of unchecked gluconeogenesis from body protein. In the normal sheep it seems probable that gluconeogenesis from absorbed lower fatty acids is responsible.

The difference between the glucose tolerance of normal and diabetic sheep lies in the blood glucose level; apparently the diabetic sheep at its high blood glucose level has a tolerance for injected glucose which is comparable to the tolerance for injected glucose exhibited by the normal sheep at its characteristic blood glucose level. This is essentially the same conclusion reached by Soskin and Levine (23) with respect to glucose utilization of diabetic dogs, namely, that the diabetic animal at its characteristically high blood glucose level utilizes as much glucose as the normal animal at its characteristic blood glucose level. A glycemia of 35 to 65 mgm. per 100 ml. is sufficient to drive an adequate carbohydrate metabolism in the normal sheep. A fourfold increase, as observed in the diabetics, would be expected to increase utilization four times in a normal sheep. Judging from the glucose tolerance, however, the glucose utilization of the diabetic sheep at this high level is similar to the normal sheep utilization at a level of 35 to 65 mgm. per 100 ml. At the same glycemic level, one would expect that the normal sheep utilizes four times as much glucose as the diabetic. A similar relationship exists between the normal and alloxan diabetic blood glucose levels of the dog, 90 to 100 mgm. per 100 ml. and 300 to 450 mgm. per 100 ml., respectively. If the glycemic level of the diabetic animal is adjusted so as to produce a normal rate of glucose utilization, one can con-



clude that the diabetic sheep exhibits the same degree of underutilization as does the diabetic dog.

A similar relationship exists between the glucose tolerance curves of fasting normal and fasting diabetic sheep, that is, the same form of curve with similar slope occurs at different blood glucose levels. A comparison of the tolerance under fed and fasting conditions, however, reveals a marked decrease in the latter. It is apparent that a condition of fasting causes physiological responses which are not evoked by the diabetic state in sheep.

It has already been mentioned that excessive gluconeogenesis from body protein is responsible for the low glucose tolerance of the fasting normal carnivore. An increased gluconeogenesis could be effected through a pathway described by Long (24), who has found that adrenaline secretion stimulates the anterior pituitary gland to secrete the adrenocorticotrophic hormones which are believed to increase gluconeogenesis, in part at least, by mobilizing body proteins and fats. Since the output of adrenaline into the blood is known to be increased during hypoglycemic states, the latter is a probable factor in controlling the gluconeogenic process. In *sheep 2* and *3*, the glycemia before glucose administration were subnormal with respect to their characteristic levels; hypoglycemia was not so apparent in the fasting diabetic tolerance study on *sheep 8*, and was absent in the study carried out in this animal before the production of diabetes. It should be pointed out that hypoglycemic symptoms are known to occur at higher blood sugar levels in diabetic animals, in which glucose utilization is low. Although it is probable that the decreased glucose tolerance of the fasted sheep is the result of mobilization of fat and, to a lesser extent, body protein, the complete mechanism which causes this mobilization cannot be identified with certainty at this time.

In order to gain an idea of the relative importance of dietary factors in diabetes in sheep and dogs, it is necessary to study the fasting values in comparison to those obtained when the animals were on full feed. Because food intake was estimated rather than measured, and also because of the variation in digestibility and caloric value of the feed, it is impossible to express the alimentary factor quantitatively.

Wastage of glucose and ketone bodies through urinary excretion continues when diabetic carnivores are fasted. Both are diminished in fasting diabetic sheep, the former to the vanishing point. This marked drop in glucose excretion must be explained by a high renal threshold, which threshold is close to that of dogs. It is possible, also, that exogenous gluconeogenesis accounts for a large proportion of the glucose excreted when the animals are fed.

It is harder to explain the decreased ketonuria. Ketosis of fasting has been observed in both dogs and humans; ketosis also occurs in pregnancy disease of ewes, which may be the result of undernutrition. However, in the fasting diabetic sheep, ketonuria was low in degree, although ketonemia continued. Thus, diabetic ketonuria like glycosuria in sheep appears to be largely of dietary origin.

In 4 out of 6 sheep, the diabetic hyperglycemia was maintained under fasting conditions. This indicates an endogenous source of carbohydrate precursors. As has been mentioned previously, the lower fatty acids are important sources in the ruminant.

In *sheep* 3 and 7, Dorset and Shropshire ewes, respectively, normal blood glucose levels were obtained when the animals were fasted. Both of these animals seemed to suffer less from diabetes than the other animals, which were Hampshires. The former animal lived 5 months without benefit of exogenous insulin, and by excessive food intake, regained the weight lost in the initial period of alloxan diabetes. *Sheep* 7 was sacrificed after 94 days of alloxan diabetes; insulin was not necessary for survival. Ketosis, as measured by the degree of ketonemia, was mild in both animals, and ketonuria decreased to normal when they were fasted; the fasting values of urine nitrogen were low. The resemblance to the Houssay preparation, the depancreatized-hypophysectomized dog, is striking. It should be pointed out that gluconeogenesis from body protein is greatly reduced after hypophysectomy; Braier (25) reported low levels of nitrogen excretion in fasting hypophysectomized dogs, similar to the levels obtained in the fasting diabetic sheep.

#### SUMMARY

Intravenous administration of alloxan monohydrate produced the typical triphasic response to alloxan injection, namely, initial hyperglycemia, secondary hypoglycemia and tertiary permanent diabetic hyperglycemia in 7 sheep which were so treated. Seventy-five mgm. of alloxan per kgm. was found to be the diabetogenic dose for sheep. Higher doses frequently cause severe renal and hepatic damage.

Severe hyperglycemia, glycosuria, ketonemia, and ketonuria developed in the 6 sheep which did not suffer renal damage. Urinary nitrogen excretion was increased almost twofold in these animals. The ketosis decreased after the first few weeks of diabetes.

Fasts of 4 to 8 days caused a diminution or disappearance of glycosuria. Ketonuria and urinary nitrogen excretion were also decreased. Ketonemia continued throughout the fast. The blood glucose level fell to normal in two instances, and in six others the reduction was of less degree.

Polyphagia caused an increase of 100 mgm. per 100 ml. of blood in the degree of hyperglycemia in one instance. The influence of absorbed lower fatty acids upon intermediary metabolism of normal and diabetic sheep is discussed. Body protein apparently is secondary as a carbohydrate source in the sheep; gluconeogenesis from dietary volatile fatty acids assumes a more important rôle in this regard. Underutilization of glucose is also prominent in diabetic sheep.

A weight loss of 10 to 17 per cent was observed in the first two weeks of alloxan diabetes. Increased food intake later compensated in part for this loss. Survival of those animals presenting cases of uncomplicated alloxan diabetes was relatively long, from 1 to 5 months, without continued insulin therapy.

The authors express appreciation to Dr. Peter Olafson and Mr. John Kent for assistance in histological and pathological studies included in this paper.

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# A COMPARISON OF GLUCOSE METABOLISM AFTER HEMORRHAGE IN NON-DIABETIC, ALLOXAN DIABETIC AND DEPANCREATIZED DOGS

CLARISSA HAGER BEATTY<sup>1</sup>

*From the Department of Physiology, College of Physicians and Surgeons, Columbia University*

NEW YORK CITY

WHEN dogs are subjected to 75 per cent hemorrhage by Walcott's method (1), their arterial blood glucose concentration rises rapidly, reaching a maximum value at varying times after hemorrhage and almost invariably showing a sharp terminal decrease (2). Furthermore the animals also show an initial increase in the A-V glucose difference (peripheral glucose utilization), followed by a terminal decrease. However, glucose utilization does not necessarily rise and fall simultaneously with the rise and fall of the arterial blood glucose level. Moreover, the intravenous injection of sufficient glucose to raise the blood sugar level of normal dogs to approximately the height caused by a 75 per cent hemorrhage does not raise the A-V difference to the high value found after bleeding. This is true even when one takes into account the differences in blood flow (3). Changes in A-V glucose difference are dependent not only upon the arterial blood glucose level and the rate of blood flow, but also upon one or more additional factors. All or nearly all the rise in the arterial blood glucose concentration after hemorrhage is caused by the secretion of epinephrine (3, 4). However, epinephrine hyperglycemia has little influence on glucose utilization (5-7), although epinephrine is known to increase the muscle plasma glucose ratio (8). These findings indicate that some factor other than epinephrine secretion—changes in the rate of blood flow, or increased arterial blood glucose level—appears to increase the glucose A-V difference after hemorrhage. Since insulin is known to increase the rate of removal of blood glucose by peripheral tissues (6, 9) the following experiments were performed to determine whether or not changes in the secretion of insulin are concerned with variations in the glucose A-V difference (glucose utilization) induced by a 75 per cent hemorrhage.

## METHOD

Animals in which the secretion of insulin was decreased or abolished were prepared by the administration of alloxan or by pancreatectomy. The use of alloxan injection rests upon the evidence that this substance specifically destroys the beta cells of the pancreas (10-13).

Seventeen dogs, weighing 7 to 13 kg., were injected intravenously with 50 to 60 mg/kg. of alloxan monohydrate dissolved in 10 cc. of distilled water. These animals were fasted for 18 to 24 hours before injection. The dogs were kept for 3 to 11 days after the injection of alloxan, blood glucose determinations being made every other day. If the blood glucose concentration did not increase above the control value or rose and returned to the preinjection level, additional injections of

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Received for publication June 10, 1948.

<sup>1</sup> Now associated with the Departments of Physiology and Biochemistry, University of Oregon Medical School, Portland, Ore.

50 to 150 mg/kg. of alloxan were given until the arterial whole blood glucose concentration stayed above 200 mg. per cent. When the animal appeared to be in a permanent state of hypoinsulinism, it was fasted for 18 to 24 hours and subjected to a 75 per cent hemorrhage by Walcott's technique (bled out and 25 per cent of the bleeding volume immediately returned).

Total pancreatectomies were made on 9 dogs weighing between 8 and 13 kg. During the four to seven days following the operation, each animal was given 3 to 10 U of protamine zinc insulin<sup>2</sup> per day. This maintained the blood glucose concentration at the pre-operative level. When the wound had healed and the animal was in good condition, insulin was discontinued and two to five days later the dog was fasted 18 to 24 hours and hemorrhaged 75 per cent of its bleeding volume.

Glucose, lactate and pyruvate analyses were made on whole blood.  $\text{ZnSO}_4$  and NaOH were used to precipitate the blood proteins for the glucose and lactate analyses. Glucose determinations were made according to Somogyi's method (14) and lactate and pyruvate analyses were carried out as previously described (2). Plasma bromsulphaleins were determined in a Coleman Junior Spectrophotometer, the color being developed according to the method given in Peters and Van Slyke (15). The thymol flocculation test was carried out on heparinized plasma as described by MacLagan (16). The liver was examined histologically for fat by staining frozen sections with Sudan IV, the tissues being previously fixed in 10 per cent formaldehyde. Plasma volume determinations were done with the blue dye, T-1824 (17). Hematocrit readings were made on heparinized blood by centrifuging for 30 minutes at 3000 r.p.m. in Wintrobe tubes.

The relative reduction in the volume of blood flowing through the hind limb following a 75 per cent hemorrhage was estimated by inserting a T-shaped cannula into the femoral vein of dogs in which the blood had been heparinized using the dosages recommended by Solandt and Best (18). After clamping the femoral vein the blood from the side arm of the cannula was collected for a measured time interval. It is recognized that this procedure measures only the relative variations in the blood flow.

## RESULTS

The injection of alloxan has been reported (12) to cause liver damage. Since the extent to which such liver damage might influence glucose metabolism is unknown, the amount of injury in the livers of the first few dogs injected with alloxan was investigated. All of the thymol flocculation tests done on 7 dogs before and after the injection of alloxan were negative (table 1), with the exception of *dog 10*, in which the test became 1+ when the animal developed distemper. Seven dogs, some of which were used for the thymol flocculation test, were injected with 4 mg/kg. of bromsulphalein. The administration of alloxan produced no change in the disappearance rate of bromsulphalein (table 1). *Dog 10*, which contracted distemper and showed a 1+ thymol flocculation test, also showed a significantly higher 60-minute plasma bromsulphalein level. The livers from 9 alloxan diabetic dogs were stained with Sudan IV and examined for fat (table 1). In 5 of these animals the fat content of the liver increased. The other 4 animals had no more fat in their liver than is often present in uninjected control animals; only an occasional parenchymal cell or cluster of cells containing fat was seen. Fat was, of course, found in the bile duct epithelium of both control and injected animals. When marked fatty infiltration occurred the fat was distributed in the area surrounding the central veins. Further liver function tests were not done because the data presented later in this paper gave no indication of any impairment in the ability of the liver to produce glucose after the administration of alloxan even in the presence of considerable fatty infiltration.

<sup>2</sup> Eli Lilly and Co. Indianapolis, Ind.

The utilization of glucose before and after a 75 per cent hemorrhage was studied on 13 alloxan diabetic and 7 depancreatized dogs. A decrease in blood glucose as the blood passed through the tissues of the hind limb (negative A-V difference) was arbitrarily termed glucose utilization. The disappearance of glucose from the blood stream may not signify tissue utilization, but as is shown later the A-V difference may be used as an index of glucose utilization under the conditions of these experiments.

TABLE 1. DOSE OF ALLOXAN GIVEN TO THE DOGS AND THE CHANGE IN THE BLOOD GLUCOSE LEVEL AFTER THE INJECTION OF THE ALLOXAN, COMPARED WITH THE AMOUNT OF LIVER DAMAGE

DOG	WHOLE BLOOD GLUCOSE IN MG. %			DOSE OF ALLOXAN	DAYS AFTER ALLOXAN BEFORE TERMINUS	LIVER FAT CONTENT	THYMOL FLOC. TEST <sup>1</sup> AFTER ALLOXAN <sup>2</sup>	BROMOSULPHALEIN LEVELS IN PLASMA AFTER 4 MG/KG. OF DYE			
	Before alloxan, control	After alloxan						Before alloxan <sup>9</sup> (mg. %)		After alloxan <sup>9</sup> (mg. %)	
		Max.	Termi- nal					10 min.	1 hr.	10 min.	1 hr.
				mg/kg.							
1	53	271	101	53 <sup>3</sup>	8	+					
2	61	298	273	50 <sup>3</sup>	37	+	neg. 8 days <sup>6</sup> neg. 25 days				
3	73		4	50	1	++++					
4	73	206	111 <sup>5</sup>	50 <sup>7</sup>	11	++	neg. 4 days <sup>8</sup> neg. 6 days				
				75							
5	60		533 <sup>5</sup>	50 <sup>7</sup>	3	+		.3	.1		
6	65	153	85	50 <sup>3</sup>	7	+	neg. 2 days neg. 4 days	.3	0	.2	0
7	71		787 <sup>5</sup>	60 <sup>7</sup>	4	+++					
8	65		359 <sup>5</sup>	55 <sup>7</sup>	5	+++	neg. 2 days neg. 3 days	.5	.1	.	.2
9	55	298	136 <sup>5</sup>	52 <sup>7</sup>	5	++	neg. 2 days	.6	.2	1.2	.4
10	55	573		55 <sup>3</sup>	6		neg. 3 days 1 + 6 days	.5	.2	.7	.2
11	73	220	197	50	20		neg. 14 days	.3	.1	.4	.1
12 <sup>10</sup>		82		50				.8	.1		

<sup>1</sup> All thymol flocculation tests before alloxan were negative. <sup>2</sup> Blood glucose above 200 mg. %.

<sup>3</sup> Experiments terminated with nembutal. <sup>4</sup> Died hypoglycemic convulsion. <sup>5</sup> Control blood sugar on day of experiment (75% hemorrhage). <sup>6</sup> Number of days after alloxan before test was performed. <sup>7</sup> Liver studied following a fatal 75% hemorrhage. <sup>8</sup> Blood glucose of 103 mg. %. <sup>9</sup> Bromsulphalein excretion tests performed on same days as thymol flocculation tests. <sup>10</sup> Dog did not develop diabetes.

Glucose A-V differences were determined by taking simultaneous (within less than 2 minutes) samples of blood from the femoral artery and vein. The individual and average arterial whole blood glucose concentrations and glucose A-V differences of non-diabetic, alloxan diabetic and pancreatectomized animals before hemorrhage are given in tables 2, 3, and 4. The arterial blood glucose levels before bleeding were higher in the diabetic than in the non-diabetic dogs. However, the pre-hemorrhagic A-V glucose differences of the three series of animals were the same.

Following hemorrhage the general pattern of glucose metabolism in the diabetic

animals was similar to that found in the non-diabetic dogs. The arterial blood glucose level rose to a maximum value. This maximum glucose concentration occurred at any time after bleeding except terminally (table 2). In the last 30 minutes of existence there was almost invariably a decrease in the glucose concentration of 10 to 110 mg. per cent. The average maximum increase after bleeding in the arterial blood

TABLE 2. RELATIONSHIP OF ARTERIAL GLUCOSE CONCENTRATION (IN MG. %) TO THE FEMORAL A-V GLUCOSE DIFFERENCE IN ALLOXAN DIABETIC AND DEPANCREATIZED DOGS BEFORE AND AFTER A 75% HEMORRHAGE

DOG	CONTROL		TIME AFTER A 75% HEMORRHAGE										SURVIVAL AFTER HEMOR- RHAGE	DOSE OF ALLOXAN	VENOUS GLUCOSE LEVEL BEFORE ALLOXAN
			1 hr.		2 hr.		3 hr.		4 hr.		5 hr.				
	Art.	A-V	Art.	A-V	Art.	A-V	Art.	A-V	Art.	A-V	Art.	A-V			
Alloxan Diabetic															
5	533	-7	668	-43	688	-8	698	-5	516				hr.	mg/kg.	60
7	787	+6	881	-96	885	-44 <sup>1</sup>							4	50	71
8	359	-8	460	-51	401	-3							1.3	60	65
13	248	-8	413	-68	416	-60	395	-48	287				2	55	90
14	247	-0	311	-25	440	-44	535	-65	551	-43			4.3	55, 55	86
15	273 <sup>2</sup>	-5	423	-47	394	-25							4.3	52	76
16	286 <sup>2</sup>	-3	670	-132	744	-172	790	-134	790 <sup>3</sup>	-94			2	53	80
17	223	-6	400	-62	419	-47	478	-50	508	-50	575	-61	3.5	53	
18	205	-1	405	-26	447	-27	498		495		582		5.5	55, 55	
											557 <sup>4</sup>		5.8	55, 80, 100, 100	72
19	252	-2	349	-30	394	-35	405	-20	442	-37	377 <sup>5</sup>	+13	6	55	83
20	233	-8												57	67
21	412	-4	610	-20	662	-16	686	-10	714	-12	640	+15	5	57	60
22	278 <sup>2</sup>	0	593	-34	575	-44	524	+2					4	55, 60, 65, 75, 100, 100, 100, 150, 150	88
23	349 <sup>2</sup>	-2	468	-40	520	-56	543	-15	501	-21			5	55	
24	312 <sup>2</sup>	0	441	-16	459	-12	492	-21	485	0			4.3	55, 55	111
Depancreatized															
1	254	-6	295	-33	336	-58	340	-52	274 <sup>2</sup>	+1			3.5		
2	303	-13	379	-30	430	-35	388	-0					2.5		
3	385	-21	555	-83	624 <sup>4</sup>	-84	608 <sup>7</sup>	-68	590 <sup>8</sup>	-57			2.5		
4	244	-12	330	-52	378	-75	412	-66	372	-41			4		
5	303 <sup>2</sup>	0	432	-49	399	-13	338	+4					3		
6	299 <sup>2</sup>	-8											2.5		
7	358 <sup>2</sup>	-3	478	-27	512	-54	450						3		
8	285	+2	405	-75	489	-77	568	-81	600	-69	552 <sup>9</sup>	-39	4.5		

<sup>1</sup> This was a 1.3-hour sample. <sup>2</sup> This animal heparinized. <sup>3</sup> This was a 3.5-hour sample. <sup>4</sup> This was a 5.8-hour sample. <sup>5</sup> This was a 6-hour sample. <sup>6</sup> This was a 1.5-hour sample. <sup>7</sup> This was a 2-hour sample. <sup>8</sup> This was a 2.5-hour sample. <sup>9</sup> 4.5-hour sample.

glucose concentrations of the alloxan diabetic animals was 109 mg. per cent greater than that of the control animals (table 3) while this value was only 52 mg. per cent for the dogs of the pancreatectomy series. Since differences in the concentrations of glucose could be explained by differences in circulatory volume produced by alloxan injection, plasma volume determinations were carried out on 6 alloxan diabetic dogs both before the injection of alloxan and either on the day preceding or on the day of the bleeding experiment. It was found that any changes in the plasma or blood

volume (calculated from dye and hematocrit readings) of the dogs after the injection of diabetogenic doses of alloxan were within the error of the method. After the administration of alloxan the plasma volume increased an average of 1.5 per cent (+8 to -9 per cent) and the blood volume decreased an average of 5.0 per cent (+4 to -11 per cent). As in the control dogs, the post-hemorrhagic femoral A-V glucose difference in the diabetic animals increased to a maximum after which a progressive

TABLE 3. WHOLE BLOOD GLUCOSE (ARTERIAL) CONCENTRATIONS AND FEMORAL A-V DIFFERENCES (MEANS) BEFORE AND AFTER 75% HEMORRHAGE

All values expressed in mg/100 cc., as mean and standard error  $\frac{s}{\sqrt{n}}$  of the mean,  $n$  = no. of animals

	CONTROL SERIES, C <sup>1</sup>	"	ALLOXAN SERIES, A	"	DEPAN. SERIES, D	"	DIFF. IN MEANS S.E. DIFF.
<i>Before hemorrhage</i>							
Blood glucose	77 ± 1.7	25	330 ± 35	17	304 ± 17	8	
A-V diff.	3.2 ± 0.5	25	3.2 ± 1.2	17	7.6 ± 2.7	8	C & A, no diff. C & D, 1.6 A & D, 1.6
<i>After hemorrhage</i>							
Max. rise above pre-hemorrhage level of blood glucose	122 ± 20	12	231 ± 32	13	174 ± 30	7	C & A, 2.9 C & D, 1.5 A & D, 1.3
Max. rise above pre-hemorrhage level of A-V diff.	33 ± 3.2	12	57 ± 11	13	55 ± 7	7	C & A, 2.1 C & D, 2.9 A & D, 0.2

<sup>1</sup> Values taken from paper of Beatty (2).

TABLE 4. AVERAGE PERCENTAGE REDUCTION IN BLOOD FLOW AFTER 75% HEMORRHAGE

	1 HOUR	2 HOURS	3 HOURS	TERMINAL <sup>1</sup>
Control (8 dogs).....	24 <sup>2</sup>	27	21 <sup>3</sup>	20
Alloxan diabetic (7 dogs).....	22	21	18 <sup>3</sup>	16 <sup>2</sup>
Pancreatectomized (3 dogs).....	25	20	20	19

<sup>1</sup> Within the last  $\frac{1}{2}$  hour of survival. <sup>2</sup> This average is for 6 dogs. <sup>3</sup> This average is for 5 dogs.

decrease occurred until death supervened. However, the average maximum femoral A-V glucose difference rose to a greater extent in the two diabetic groups than in the control, non-diabetic series (table 3). In 2 dogs with alloxan diabetes and 2 pancreatectomized animals the terminal glucose A-V difference was positive. No positive terminal A-V glucose differences were observed in any of the 11 dogs of the control series. It is interesting to note that positive A-V glucose differences have been observed in intravenous glucose tolerance curves in normal animals (3, 19). Glucose A-V differences were not corrected for changes in the hematocrit reading as the blood flowed through the leg, for the diabetic A-V hematocrit differences were small,



amounting to 0 to 2.1 units, and were not changed by hemorrhage (six determinations). Similar results have been reported previously for non-diabetic dogs (2).

Differences in the glucose A-V differences produced in the three series of animals by bleeding could be caused by variations in blood flows. For this reason, the relative change in the blood flow through the femoral vein following a 75 per cent hemor-

Fig. 1. SHOWING THE SIMILARITY of the patterns of changes in arterial blood glucose concentration and glucose A-V differences following a 75 per cent hemorrhage in control, alloxan diabetic and pancreatectomized animals. Unbroken lines represent arterial blood glucose concentration and broken lines, glucose A-V differences.

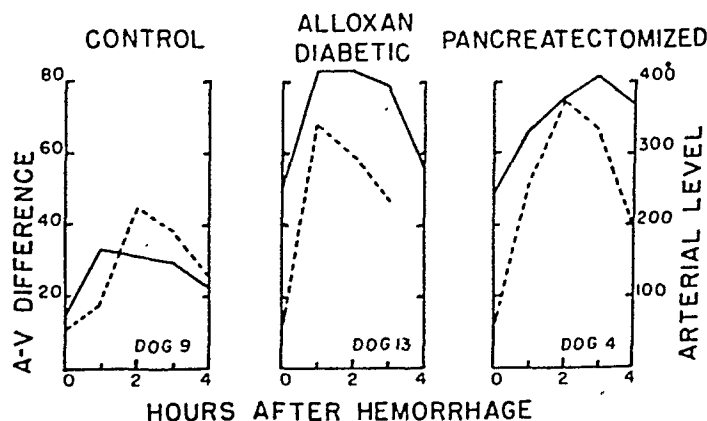


TABLE 5. COMPARISON OF WHOLE BLOOD LACTATE, PYRUVATE AND L/P RATIOS IN CONTROL, ALLOXAN DIABETIC AND PANCREATECTOMIZED DOGS, BEFORE AND AFTER A 75% HEMORRHAGE

All values expressed in mg/100 cc., as mean and standard error o/(m) of the mean. n = no. of animals

SERIES	BEFORE HEMORRHAGE			1 HR. AFTER HEMORRHAGE			TERMINAL <sup>1</sup>		
	Lactate	n	Diff. of means S.E. diff.	Lactate	n	Diff. of means S.E. diff.	Lactate	n	Diff. of means S.E. diff.
Control, C Alloxan, A Depancrea- tized, D	mg. %			mg. %			mg. %		
	12 ± 1.4	36		58 ± 3.0	36		109 ± 4.3	35	
	12 ± 1.5	6	C & A, no diff.	51 ± 6.7	7	C & A, 1.9	118 ± 6.0	7	C & A, 1.2
	27 ± 2.4	3 <sup>2</sup>	C & D <sup>2</sup>	63 ± 10.7	5	C & D, 0.45	120 ± 16.2	5	C & D, 0.7
Control, C Alloxan, A Depancrea- tized, D	Pyruvate			Pyruvate			Pyruvate		
	1.4 ± 0.14	31		4.6 ± 0.20	21		4.2 ± 0.20	29	
	1.7 ± 0.19	11	C & A, 1.2	3.2 ± 0.35	10	C & A, 3.5	4.7 ± 0.27	11	C & A, 1.5
	1.7 ± 0.04	5	C & D, 2.1	2.9 ± 0.24	5	C & D, 5.4	5.1 ± 0.44	5	C & D, 1.9
Control, C Diabetic, D	L/P			L/P			L/P		
	8 ± 2.3	31		12 ± 0.7	21		29 ± 1.6	27	
	11 ± 1.4	6 <sup>1</sup>	C & D, 1.1	20 ± 2.0	9 <sup>4</sup>	C & D, 3.8	25 ± 2.3	9 <sup>4</sup>	C & D, 1.5

<sup>1</sup> Within the last 30 min. of survival. <sup>2</sup> Too short a series to show significant difference. <sup>3</sup> Combined series consisting of 4 alloxan diabetic and 2 pancreatectomized dogs. <sup>4</sup> Combined series of 5 alloxan diabetic and 4 pancreatectomized dogs.

rhage was measured in all the series. In the animals of all three groups the limb blood flows decreased immediately after bleeding to an average of 22 to 25 per cent of the pre-hemorrhagic values (table 4). No further marked change in blood flow occurred unless determinations were made within the last 10 minutes of survival when further rapid decreases in flow were observed. When the blood flow through the femoral vein was calculated as cc/kg. of body weight, it was found that 10 control

dogs had an average flow of 4.2 cc/kg., 6 alloxan diabetics 4.4 cc/kg. and 3 depancreatized 4.3 cc/kg. These results indicate that differences in the peripheral rate of disappearance of glucose between the control and the two diabetic series cannot be related to differences in the blood flow through the hind limb. Crandall and Lipscomb (20) likewise found little change in the blood flow through the liver of dogs after pancreatectomy.

Before hemorrhage the average blood lactate and pyruvate values and L/P ratios in the control, alloxan diabetic and depancreatized dogs showed no differences, except for the increased lactate concentration of the depancreatized animals (table 5). Since lactate and pyruvate determinations were not always made on the same animals, the L/P ratios of the two diabetic groups were combined in order to obtain a number large enough for comparison with those of the control group. The rise in blood lactate concentrations after hemorrhage (table 5) was similar in the three groups of animals. The blood pyruvate levels also rose (table 5), but the concentrations the first hour after bleeding were lower in the alloxan diabetic and depancreatized series than in the control series. At this time the average L/P ratio of the combined diabetic group was higher than that of the control series. The average terminal blood pyruvate levels were not different in the three series of animals, nor was there any difference between the terminal L/P ratios of the combined diabetic and control groups.

Comparison of the bleeding volumes in cc/kg. shows that there were no differences between the values obtained on the control animals and those of the two diabetic series (table 6). The bleeding volumes were calculated on the basis of the dog's weight on the day of the experiment. The average survival time of the three series of animals was also similar,  $3\frac{3}{4}$  hours for the control group, 4 hours for the alloxan diabetics and  $3\frac{1}{4}$  hours for the depancreatized.

Since anemia has been reported in man after alloxan injection (21), hematocrit determinations were made on 10 dogs before and after the injection of varying doses of alloxan (table 7). In 9 of 10 animals the hematocrit values decreased. The total circulating red cell volume was calculated for 7 dogs in which plasma volume had been determined (table 7). In 6 of 7 dogs a decrease of 3 to 20 per cent (average 14 per cent) in the total circulating red cell volume was found.

#### DISCUSSION

There were no significant differences in the average pre-hemorrhagic femoral A-V glucose differences (table 3), nor in the relative blood flows of the alloxan diabetic, depancreatized and control series. These findings indicate, in agreement with Soskin and Levine (22), that, on the average, the blood glucose level in our diabetic animals was high enough to enable the muscles of the hind limbs to utilize sugar in approximately the same amount as in the control, non-diabetic animals.

Statistically speaking, the increase in the arterial blood glucose level of the hemorrhaged depancreatized dogs was equivalent to that of the control dogs, whereas the rise in glucose concentration in the animals of the alloxan diabetic series was greater than that in the control group (table 3). A possible difference between the alloxan diabetic and the depancreatized dogs may be explained by the less favorable physical

condition of the depancreatized animal in comparison with the alloxan dog. Pancreatectomy caused a greater drop in weight than injection of alloxan, although the two series of dogs were diabetic for similar periods of time. However, the differences in weight loss between the two diabetic groups was not significant (table 6). Another explanation for a difference between the two diabetic series may be the presence of a small number of functioning beta cells in the alloxan diabetic dog. A third explana-

TABLE 6. COMPARISON OF BLEEDING VOLUMES AND SURVIVAL TIMES IN CONTROL, ALLOXAN DIABETIC AND DEPANCREATIZED DOGS UNDERGOING A HEMORRHAGE OF 75% OF THE BLEEDING VOLUME

All values expressed in mg/100 cc., as mean and standard error o/(vn) of the mean. *n* = no. of animals

SERIES	BLEEDING VOL.	<i>n</i>	DIFF. OF MEANS S.E. DIFF.	WT. LOSS	<i>n</i>	DIFF. OF MEANS S.E. DIFF.	SURVIVAL TIME	<i>n</i>	DIFF. OF MEANS S.E. DIFF.
	cc/kg.			kg.			hr.		
Control, C	60 ± 1	72		0.5 ± 0.2 <sup>1</sup>	21		3.75 ± 0.3	14	
Alloxan, A	54 ± 3	14	C & A, 1.8	0.9 ± 0.1 <sup>2</sup>	13	C & A, 1.6	4.00 ± 0.4	15	C & A, 0.5
Depancreatized, D	56 ± 3	8	C & D, 0.9	1.4 ± 0.4	6	C & D, 2.2	3.25 ± 0.3	8	C & D, 1.2

<sup>1</sup> Weight loss during the 18- to 24-hr. fast preceding the experiment. <sup>2</sup> Weight loss during the entire diabetic period, including the 18- to 24-hr. fast preceding the experiment.

TABLE 7. HEMATOCRIT VALUES AND TOTAL CIRCULATING RED-CELL VOLUMES BEFORE AND AFTER THE INJECTION OF DIABETOGENIC DOSES OF ALLOXAN

DOG	HCT. BEFORE ALLOXAN	HCT. AFTER ALLOXAN	CHANGE IN HCT.	% CHANGE IN TOTAL RED-CELL VOL. <sup>1</sup>	DOSE OF ALLOXAN	DAYS BETWEEN 1ST ALLOXAN AND HCT. DETER.
					mg./kg.	
25	50.5	46.0	-4.5	-20	55, 55	6
18	34.0	26.0	-8.0	-10	55, 80, 100, 100	9
26	46.3	40.7	-5.6	-19	55, 60	5
20 <sup>2</sup>	37.6	49.5	+11.9		57	6
21	47.7	41.1	-6.6	-3.0	57	3
22 <sup>3</sup>	42.6	41.7	-0.9		3.3 gm. <sup>4</sup>	15
23	47.5	41.0	-6.5	-17	55	3
24	41.7	41.5	-0.2	+2	55, 55	3
27	38.0	32.0	-6.0	-14	55	3
19	38.9	35.7	-3.2		55	7

<sup>1</sup> No correction made for relatively constant errors of trapped plasma and buffy coat. <sup>2</sup> Animal vomited frequently and did not eat well. <sup>3</sup> Animal vomited 3 times immediately following injections of alloxan but ate well. <sup>4</sup> Total alloxan over a period of 13 days.

tion might be that some pancreatic cells other than the beta cells are secreting a hypothetical second pancreatic hormone (23) that is present only in the alloxan diabetic dogs in which the beta cells are destroyed without permanent damage to other islet cells. Since the number of dogs in the depancreatized series was small for statistical purposes and the range of values large and since there was no significant difference between the post-hemorrhagic increments in arterial blood glucose levels of the two diabetic groups, all the diabetic dogs have been combined for comparison with the

control animals. When this is done a statistically significant difference can be demonstrated between the control and combined diabetic series (diff. in means/S.E. diff. = 2.9). The greater post-hemorrhagic rise in arterial blood glucose level in the alloxan diabetic dogs when compared with the control dogs cannot be explained on the basis of a decreased plasma or blood volume, because diabetogenic doses of alloxan produced no significant change in the plasma or blood volume. Therefore, the greater rise in blood glucose was probably caused either by the liver producing more glucose or by the tissues removing less glucose, or by a combination of both processes.

The average maximum femoral A-V glucose difference rose to a greater extent after hemorrhage in the two diabetic than in the non-diabetic series (table 3). It is somewhat doubtful whether the increment in the A-V difference of the alloxan diabetic dogs is statistically greater than that of the control dogs. However, the rise in the A-V difference of the depancreatized animals is clearly larger than that of the non-diabetic dogs. For the reasons stated above a comparison was made of the combined diabetic and control series and again a statistically significant difference between the two series was demonstrated (diff. in means/S.E. diff. = 3.8). The difference between the post-hemorrhagic A-V differences cannot be explained by differences in the blood flow through the hind limb because the relative changes in the blood flow through the hind leg after bleeding were similar in the control, alloxan diabetic and depancreatized dogs (table 4). A-V differences measure the disappearance of glucose from the blood stream and not necessarily utilization. The A-V difference in these experiments indicates that glucose is continually leaving the blood stream. Since equilibrium is rapidly attained, the glucose must be leaving the interstitial fluid compartment and entering the cells, in order to maintain the concentration in the interstitial fluid compartment close to the blood level. Storage of glucose as glycogen in the cells appears impossible in view of Tachi's finding (24) that severe hemorrhage causes a marked decrease in the muscle glycogen of non-diabetic rabbits. Furthermore, the rate of glycogen synthesis in the muscles, and especially synthesis from glucose, is decreased in animals which lack sufficient insulin (25, 26).

The stimulating effect on glucose utilization of the greater post-hemorrhagic increase in the blood glucose level of diabetic animals when compared with non-diabetic animals might explain the greater A-V difference in the alloxan diabetics. However, raising the arterial blood glucose concentration by intravenous injection of glucose in non-diabetic dogs increased the A-V difference less than increasing the arterial blood glucose level the same amount by hemorrhage (taking blood flow into account) (3). Intravenous glucose injections were used because ingestion by mouth raised the blood glucose level too slowly. Injection of 0.75 mg/kg. of glucose by vein increased the blood glucose concentration to about the same level as that reached by hemorrhage in a similar interval of time. As in the control series, the glucose A-V difference did not necessarily follow the arterial blood glucose concentration (for example see dog 22, table 2). Insufficient oxygen for utilization could be the direct cause of a decrease in the peripheral glucose utilization even though the arterial blood glucose level was rising, but an additional mechanism is necessary to explain how the A-V glucose difference can increase while the arterial blood glucose level is decreasing.

The fact that following hemorrhage the glucose A-V difference increased more in

the diabetic dogs than in the non-diabetic dogs may also be explained by the larger rise in the blood glucose level of the diabetic animals, increasing the amount of glucose necessary to establish equilibrium between the blood and tissues and thus enlarging the A-V difference. During periods of rapid fluctuation in glucose concentration the A-V difference may largely reflect attainment of equilibrium. However, in these experiments the glucose A-V difference often remained at high levels for an hour or more with very little change in the arterial blood glucose concentration (table 3, *dog 1*; table 2, *dog 13*). Glucose equilibrium between blood and muscles in non-diabetic rats is reached in three minutes (27). In dogs the volume into which an intravenous dose of 0.75 gm/kg. of glucose must distribute itself five minutes after injection in order to raise the arterial blood glucose concentration the required amount can be calculated (ignoring urine loss and utilization). The results indicate that the glucose has distributed itself in approximately 50 per cent of the total body weight of the dog (3). This value is close to the figure of 65 to 66 per cent of the body weight found by Painter (28) for the total body water of dogs. The fact that five minutes after injection of 0.75 gm/kg. of glucose into control dogs the glucose A-V difference was often positive and was not significantly different from the pre-injection A-V difference (3) furnished additional evidence that glucose equilibrium between blood and tissues is rapidly achieved. It is recognized that glucose does not distribute itself in equal concentrations in all the tissues (27, 29).

Because an increase in the glucose A-V difference occurred in the absence of insulin and because this increase cannot be explained entirely by changes in the blood flow, in the arterial blood glucose level or by the achievement of equilibrium, an additional mechanism must be present which, following hemorrhage, influences glucose utilization. No explanation is offered for the larger glucose A-V difference after bleeding in the depancreatized dogs and the alloxan dogs, as compared with the non-diabetic controls.

Thus, following hemorrhage, glucose disappeared in the periphery of the diabetic dogs at a faster rate than in the control dogs. Furthermore, bleeding the diabetic series increased the arterial blood glucose level more than bleeding the control series. Where is the source of the extra glucose in the diabetic series? As the liver glycogen stores in a diabetic animal tend to be low (30-32), especially following an 18- to 24-hour fast, simple calculation indicates that considerable gluconeogenesis must go on in these diabetic animals to raise and maintain the blood glucose concentration at the high level found. For example, if we assume the pre-hemorrhagic liver glycogen level of the postabsorptive diabetic dog to be approximately one per cent (Fisher and Lackey (30) give a value of 0.05 per cent for dogs on a mixed diet) and the liver to be 3.5 per cent of the body weight (32), in a 10 kg. dog, we would have roughly 3.5 gm. of glycogen in the liver which could be released as glucose after hemorrhage. Distributed in an extracellular volume comprising 25 per cent of the body weight (17), 3.5 gm. of glucose would only be sufficient to increase the arterial blood glucose level 150 mg. per cent providing no glucose disappeared from the extracellular compartment. The average rise in the blood glucose concentration in the alloxan diabetic dogs was 231 mg. per cent and glucose was disappearing rapidly into the peripheral tissues. Loss of glucose in the urine may be disregarded, because urine formation is

at a minimum following severe hemorrhage. Even if there is no increase in gluconeogenesis in diabetes under control conditions (26, 20) a severe stress, such as a hemorrhage consisting of 75 per cent of the bleeding volume, might cause more gluconeogenesis in diabetic than in non-diabetic dogs. It is possible that gluconeogenesis is the source of the extra glucose found in the two diabetic series after bleeding.

Statistically speaking there was no significant difference in the average pre-hemorrhagic blood lactate and pyruvate values and L/P ratios in the three groups of animals, except for the higher lactate concentration of the depancreatized dogs (table 5). In view of the fact that this series consisted of only 3 animals and that Chesler and Himwich (34) reported no change in the lactate and pyruvate levels of their depancreatized dogs, these elevated lactate concentrations probably should not be considered significant. One hour after bleeding there was no significant difference in the average lactate values in the three series. The two groups of diabetics had a significantly lower pyruvate level and higher L/P ratio than those of the control dogs. Terminally the average lactate, pyruvate and L/P values were similar for the three series of animals.

#### SUMMARY

1. The glucose metabolism in a series of alloxan diabetic and a series of depancreatized dogs was investigated before and after a 75 per cent hemorrhage (bled out and 25 per cent of the bleeding volume immediately returned) and compared to a series of control animals similarly bled.

2. Glucose utilization before bleeding, as measured by femoral A-V glucose differences, was found to be approximately the same in the two diabetic series as in the control series.

3. Following hemorrhage the average maximum increment in the arterial blood glucose level was twice as large in the alloxan diabetic animals as in the control animals. The arterial blood glucose concentration of the depancreatized dogs after bleeding rose an average maximum value of 52 mg. per cent more than in the control dogs but the difference between the two series was not statistically significant.

4. The rate of disappearance of glucose in the periphery, as measured by femoral A-V glucose differences and blood flows, increased more following bleeding in the alloxan diabetic and depancreatized series than in the control series. Statistically speaking the difference between the diabetic and the non-diabetic dogs was somewhat doubtful in the case of the alloxan animals but was of definite significance in the case of the pancreatectomized animals.

5. Glucose disappeared in the periphery of the depancreatized dogs and perhaps also in the alloxan diabetic dogs at a faster rate than in the control animals following a hemorrhage of similar severity. Furthermore the posthemorrhagic arterial blood glucose level, at least in the alloxan diabetic animals, was raised to a significantly higher concentration than in the control dogs. Hepatic gluconeogenesis was indicated as the probable source of the extra glucose appearing in the diabetic animal after hemorrhage.

6. Lack of insulin made no difference in the general pattern of arterial blood glucose concentration and utilization after bleeding. In both the diabetic and the

non-diabetic groups the arterial blood glucose concentration rose with hemorrhage and there was almost invariably a decrease in the concentration within the last 30 minutes of survival. The glucose utilization also rose to a maximum after bleeding and this maximum was followed by a progressive decrease until death supervened.

The author is indebted to Dr. Walter S. Root and Dr. James B. Allison for their valuable assistance in the preparation of this manuscript and to Dr. J. W. Fertig for aid with the statistical treatment.

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# DESOXYCORTICOSTERONE ACETATE AND BLOOD PRESSURE OF DOGS ON A HIGH SODIUM CHLORIDE INTAKE

JOHN E. SUMMERS<sup>1</sup>

*From the University of South Dakota, School of Medicine*

VERMILLION, SOUTH DAKOTA

THE production of hypertension in patients with Addison's disease during treatment with desoxycorticosterone acetate (DCA) (1-3) has stimulated attempts to produce hypertension in subjects with normal blood pressures by the administration of the hormone (2, 4). Perera and Blood (4) found that the blood pressures of 10 normotensive subjects were not affected by the administration of 5 mg. of DCA given subcutaneously twice daily for one week, with the patients receiving 5 to 10 grams of NaCl orally each day. On the other hand, these investigators (4) found that the blood pressures of 14 subjects with uncomplicated hypertensive vascular disease were increased under the same conditions.

Favorable reports (5) on the treatment of hypertension with a diet poor in sodium have stimulated research in the direction of the adrenal cortical hormones and their possible rôle in the production of essential hypertension.

It has been shown that the parenteral administration of DCA in dogs produces a diabetes-insipidus-like syndrome, i.e., polydipsia and polyuria (6, 7). Selye and associates (8-12) have produced malignant hypertension in rats on a high NaCl intake by the subcutaneous administration of 3 mg. of DCA twice daily for the first month and 5 mg. of DCA twice daily during the second month of treatment. Selye and Hall (8), using 2 recently weaned puppies, administered 5 mg. of DCA subcutaneously twice daily for one week, 10 mg. twice daily for the second week and 20 mg. twice daily for the remainder of the experiment. The female animal received 1530 mg. of DCA over a 47-day period; the male received 2450 mg. over a 70-day period. During the period of administration of the DCA paralysis of the neck and shoulder muscles could be produced by giving the dogs NaCl. The effect on the blood pressure was not noted. Autopsy of these 2 puppies revealed that the kidneys were enlarged and pale, the convoluted tubules were dilated and a proliferation of cells had occurred within the glomeruli. The hearts were enlarged and pale. The adrenal glands, both the cortex and the medulla, showed an extreme degree of atrophy. The liver, pancreas, thyroid, parathyroid and pituitary glands showed no appreciable change grossly or microscopically.

According to Durlacher and Darrow (13), a low potassium diet in the rat causes hypertrophy of the kidney and dilatation, hypertrophy and hyperplasia of the loops

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Received for publication May 21, 1948.

<sup>1</sup> Now Resident in Orthopedic Surgery, The Norwalk Hospital, Norwalk, Conn.



of Henle and of the collecting tubules. Similar though less marked changes were produced by the administration of DCA to rats on a normal diet.

#### METHOD

Six adult, healthy-appearing, mongrel dogs were used. The dogs received an adequate diet and raw meat twice weekly. All dogs were given a two-month acclimatization period before the experiment was begun. During this acclimatization period the weights were checked daily and blood pressure determinations were obtained two or three times weekly. The method of determining the blood pressure was by direct femoral artery puncture using a mercury manometer.

Two dogs were used as controls, 2 received, in addition to the regular diet, 10 grams of NaCl daily, and 2 dogs received the regular diet, 10 grams of NaCl daily and intramuscular injections of DCA.

#### *Control Animals*

*Animal no. 5 (male).* From February 9, 1948 to May 12, 1948 the weight gradually rose from 44 to 51 pounds. In a total of 18 determinations the blood pressure varied between 115 and 130 mm. Hg, and averaged 125 mm.

*Animal no. 6 (male).* From March 19, 1948 to May 12, 1948 the weight varied between 45 and 48 pounds. In a total of 18 determinations the blood pressure ranged between 110 and 120 mm. Hg, with a mean of 113 mm.

#### *Animals Receiving 10 Grams of NaCl Daily*

The oral administration of NaCl, in tablet form, 10 grams daily, was begun on March 17, 1948 and continued to May 12, 1948.

*Animal no. 2 (female).* From February 4, 1948 to May 12, 1948 the weight gradually rose from 41 to 47 pounds. In a total of 19 determinations the blood pressure varied between 120 and 130 mm. Hg, and averaged 125 mm.

*Animal no. 4 (male).* From February 9, 1948 to May 12, 1948 the weight gradually rose from 58 to 64 pounds. In a total of 18 determinations the blood pressure varied between 110 and 130 mm. Hg, with a mean of 115 mm.

#### *Animals Receiving NaCl and DCA*

These dogs received 10 grams of NaCl in tablet form daily from March 17, 1948 to May 12, 1948. Each animal also received, by intramuscular injections, the following doses of DCA dissolved in peanut oil: from April 5, 1948 to April 28, 1948, 50 mg. daily; from April 28, 1948 to May 11, 1948, 100 mg. daily; on May 11, 1948 each animal received 500 mg. of the hormone. Each animal received a total of 2000 mg. of DCA over approximately a one-month period.

The only reaction to the hormone that was manifested clinically was that for one day, April 23, 1948, when both dogs developed violent, generalized tremors with weakness of the extensor muscles of the head. During the period of administration of DCA both animals lost their appetite for the dog biscuits and were fed raw meat daily.

*Animal no. 1 (female).* From February 4, 1948 to May 12, 1948 the weight varied from 34 to 38 pounds. In a total of 18 determinations the blood pressure varied between 115 and 140 mm. Hg. The higher readings (above 130 mm. Hg) were taken on this animal during the period of acclimatization before the injections of DCA were begun. The mean of the blood pressure determinations during the period of DCA treatment was 129 mm.

*Animal no. 3 (female).* From February 4, 1948 to May 12, 1948 the weight varied from 37 to 40 pounds. In a total of 19 determinations the blood pressure varied between 105 and 125 mm. Hg, averaging 112 mm. during the period of treatment.

#### SUMMARY

The blood pressures and weight of 2 dogs on a high NaCl intake were not affected by the intramuscular injections of large quantities of DCA given over a period of one month, each animal receiving a total of 2000 mg. of DCA.

The DCA and peanut oil were furnished through the courtesy of Dr. Kenneth W. Thompson of Roche-Organon, Inc., Nutley, New Jersey.

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# STABILITY OF PROTHROMBIN AND AC-GLOBULIN IN STORED HUMAN PLASMA AS INFLUENCED BY CONDITIONS OF STORAGE<sup>1</sup>

JOHN L. FAHEY, ARNOLD G. WARE, AND WALTER H. SEEGER

*From the Department of Physiology, Wayne University College of Medicine*

DETROIT, MICHIGAN

THE realization that normal plasma contains a factor which affects the transformation of prothrombin to thrombin (1-5) has made advisable a study of possible changes in this plasma protein occurring in stored human plasma. Because of the close relationships of this plasma-clotting accelerator substance Ac-globulin to prothrombin a study of prothrombin stability was also undertaken.

The methods of analysis for prothrombin, which formed the basis of previous reports on prothrombin stability, are now seen to be sensitive to factors other than prothrombin. Consequently studies previously made on prothrombin concentration were not always accurate. In fact none of the previous reports on prothrombin stability in stored human plasma appear to have truly represented the changes of prothrombin activity that occur. The prothrombin activity was followed in this investigation by means of an improved technic, specific for prothrombin. The results of this method indicate a high degree of stability to be possessed by prothrombin in stored human plasma. Also, the changes in plasma Ac-globulin on storage were followed by means of a method believed to be specific for this coagulation factor. Ac-globulin was found to be less stable than prothrombin and to be more sensitive to various conditions of storage. Indeed, for accurate representation of the stability of the components of coagulation, the conditions of collection and of storage must be clearly defined.

It was found in this study that not only does the nature of the anticoagulant influence the stability of Ac-globulin but so also does the anticoagulant concentration. Moreover, a factor contained in platelets was found to be concerned specifically with the rate of Ac-globulin inactivation in stored plasma. The quantity of this factor present in stored plasma is dependent on such conditions as the centrifugation intensity, used to separate the formed elements from the plasma, and upon the type of container surface utilized for collection and storage.

## METHODS

To obtain blood samples for consistent and accurate prothrombin and Ac-globulin analysis it is advisable to reduce to a minimum any possible contamination by tissue juices. Blood was drawn from the antecubital vein of normal human subjects. The syringe containing the first few cc. of blood was removed from the needle and replaced by a fresh syringe containing the anticoagulant. Unless otherwise indicated one part of 1.85 per cent potassium oxalate (0.10 M  $K_2C_2O_4 \cdot H_2O$ ) was added

Received for publication June 14, 1948.

<sup>1</sup> Aided by a grant from the United States Public Health Service.

to seven parts whole blood or two parts of 3.2 per cent sodium citrate (0.109 M  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) to 23 parts whole blood. When the desired volume of blood had been obtained the needle was removed from the vein and the blood mixed with the anticoagulant in the syringe. This fluid was ejected gently from the syringe into a glass container and placed in an ice bath until centrifugation. The whole blood was centrifuged and the plasma removed, with care taken that none of the precipitated cellular elements be included in the plasma. This plasma was placed in a stoppered glass container and stored in the refrigerator at 3 to 5° C.

Plasma samples to serve as controls were removed at this point and frozen at -20° C. A sufficient quantity for one analysis was placed in each of a number of small test tubes which were stored in a deepfreeze until the time for analysis, when one was removed and thawed. Clean dry glassware was used except in the silicone experiments.

The determination of prothrombin activity was done by two methods. In the first of these, the 2-stage method (6, 7), the plasma is defibrinated by the addition of purified thrombin and the resultant clot removed. This defibrinated plasma is diluted with physiological saline, recalcific in the presence of an excess of thromboplastin and added to a standard fibrinogen solution so that the amount of thrombin formed will give a clotting end point in the approximate range of 15 seconds. The second method was a modification (8) of the 2-stage test previously described. The modification consists merely in substituting bovine serum (diluted 1:600 in the final reaction mixture) for the saline dilution of the defibrinated plasma. This modification is believed to supply sufficient Ac-globulin for full prothrombin conversion. Bovine serum contains a fairly stable and high Ac-globulin concentration (9). Determinations by the unmodified and modified 2-stage methods were made simultaneously.

Ac-globulin concentration was measured by the method of Ware and Seegers (5). The prothrombin, thromboplastin and calcium concentrations are controlled, leaving the rate of thrombin production proportional to the amount of Ac-globulin in the plasma sample to be tested.

#### EXPERIMENTAL

*Prothrombin.* To study the stability of prothrombin both citrate and oxalate were utilized individually as anticoagulant mediums. Blood was taken at the same drawing into separate syringes containing the anticoagulants. Centrifugation was carried out at 3000 r.p.m. for 20 minutes and the plasma portion preserved.

In citrated plasmas, as is indicated in figure 1A, the prothrombin activity remained unchanged for 8 to 10 days. Both the modified and unmodified 2-stage methods of prothrombin analysis gave similar results. Yet as storage was prolonged beyond 10 days the prothrombin titer, as determined by the original 2-stage method, began gradually to fall. This is an apparent reduction of the prothrombin level, for it did not occur in the measurements by the modified 2-stage analysis (fig. 1A). The modified 2-stage procedure provides Ac-globulin for complete prothrombin conversion to thrombin. This is not assured in the original method. For this reason we believed that the apparent decrease shown by the original method is due to an altera-

tion of some other constituent in the plasma than prothrombin, presumably Ac-globulin.

A more striking comparison of the two methods was afforded by the change appearing in oxalated plasma. In figure 1B is seen the marked decline in prothrombin activity evidenced by the unmodified 2-stage analysis. However, here again no prothrombin change was seen when an adequate supply of accelerator substance is present as in the improved 2-stage technic.

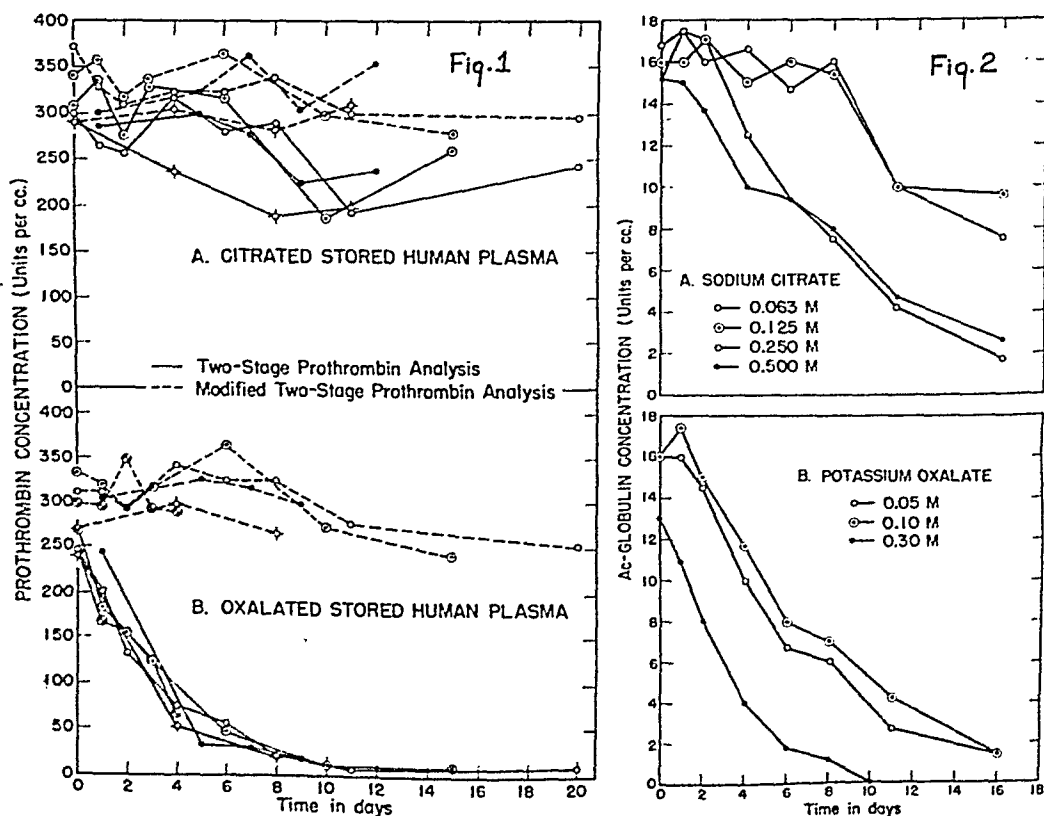


Fig. 1. PROTHROMBIN ACTIVITY in stored human plasmas decalcified with sodium citrate (A) and potassium oxalate (B) at standard concentrations. Prothrombin determinations were made by the original 2-stage method of analysis and by the modified 2-stage technic.

Fig. 2. EFFECT OF ANTICOAGULANT CONCENTRATION on Ac-globulin stability in stored human plasma. One part of anticoagulant at the concentration given was added to nine parts of whole blood.

It seems pertinent to mention at this point that in the plasma samples which were also followed by means of Quick's 1-stage test (10, 11), a stability difference was observed depending upon whether oxalate or citrate was used as anticoagulant. The observed increase in the prothrombin time in oxalated plasmas agreed with the observations of that author. However, no prothrombin time increase occurred in the citrated plasmas until after the fifth day of storage, and then did not progress so rapidly as the change which had appeared in the oxalated plasma.

In another experiment to be discussed more fully later stability studies were carried out for as long as 56 days (fig. 4) with no alteration in the prothrombin concentration evident when the analyses were done by the modified 2-stage method.

By the old technic the apparent prothrombin titer of oxalated plasma fell to a minimum of 3 to 5 per cent of the original concentration and was maintained at that level. In citrated plasma the decrease did not begin so soon nor was it as marked as that which occurred in oxalated plasma.

Thus it is evident that there is a difference in the stability of the stored coagulation factors. The activity of the less stable protein would seem to depend upon whether citrate or oxalate is utilized to maintain the plasma in the fluid state. Moreover, these alterations are not attributable to changes of prothrombin itself for, if the activity of this plasma fraction is accurately measured such as by the modified 2-stage technic, no decrease was found in 56 days under storage conditions in either citrated or oxalated human plasma.

To further examine the reason for the discrepancy between the original and the modified 2-stage procedure the following experiment was undertaken. An oxalated sample of human plasma stored in the refrigerator for 53 days and containing no detectable Ac-globulin was used. Prothrombin determinations were carried out by the original and by the standard modified 2-stage procedure. In addition, tests were

TABLE 1. PROTHROMBIN ACTIVITY IN OXALATED HUMAN PLASMA STORED 53 DAYS

METHOD OF PROTHROMBIN ANALYSIS	PROTHROMBIN CONCENTRATION <sup>1</sup>
	U/cc.
1. 2-stage unmodified. . . . .	19
2. 2-stage modified by use of bovine serum. . . . .	288
3. 2-stage modified by use of purified Ac-globulin. . . . .	295
4. 2-stage modified by use of bovine platelet extract. . . . .	285

<sup>1</sup> Original prothrombin concentration = 290 U/cc.

carried out by modifications of the 2-stage method in which a purified serum type Ac-globulin (9) or a potent bovine platelet extract was substituted for bovine serum as the diluent for the defibrinated plasma. As can be seen in table 1 the very low value found by the original technic is in sharp contrast to the full prothrombin titer that was achieved with all three of the modifications of the prothrombin test. Whether bovine serum or a purified product is the source of Ac-globulin does not appear to be of importance. Nevertheless the concentration of accelerator factor must reach at least a certain level or the conversion will be incomplete, and an inaccurate, low-prothrombin value will be obtained. A factor in platelets which affects prothrombin conversion to thrombin has recently been studied extensively in this laboratory. This factor is described as having much the same function as Ac-globulin. Here is demonstrated, with the use of stored plasma, the ability of the platelet factor to accelerate and cause complete conversion of prothrombin to thrombin.

*Ac-globulin.* The study of the stability of coagulation factors in human plasma was extended to Ac-globulin. Here again both oxalated and citrated plasmas were used (fig. 3A). Ac-globulin was found to be relatively stable in citrated plasma, maintaining full potency for more than a week before entering upon a period of gradual decline. Oxalated human plasma on the other hand did not seem to provide

a suitable medium for preservation of Ac-globulin activity. Thus at the concentrations used oxalate and citrate differ in their affect on the stability of what appears to be a single plasma fraction, Ac-globulin. The similar results obtained with the Ac-globulin assays and the unmodified 2-stage method of prothrombin analysis (fig. 3 and 4) substantiate further the conclusion that the latter test is sensitive to a fall in Ac-globulin when the human prothrombin/Ac-globulin ratio passes a certain critical value.

*Anticoagulant Concentration.* Ac-globulin sensitivity in stored human plasma to the factors affecting its stability indicate that these conditions needed to be further defined. In the previous studies 0.109 M sodium citrate was combined with whole blood in the ratio of two parts to 23. When potassium oxalate was used as anticoagulant one part of a 0.10 M solution was added to seven parts of whole blood. To further investigate the effects of these decalcifying agents sodium citrate was prepared in 0.50, 0.25, 0.125 and 0.063 M concentrations and potassium oxalate was made up in 0.30, 0.10 and 0.05 M solutions. To 1 volume of each anticoagulant concentration were added 9 volumes of blood, all samples being obtained at one drawing of blood. After centrifugation for 30 minutes at 3000 r.p.m. the plasma was removed and stored in the refrigerator. The stability of Ac-globulin as observed in these plasmas is graphically represented in figure 2.

This is additional evidence that Ac-globulin is more stable in citrated than in oxalated plasma depending, however, on the concentration of anticoagulant used. It is evident that at the lower concentrations the Ac-globulin is more stable in both mediums. Interestingly enough the plasma stability curves from the bloods mixed with the higher concentrations of sodium citrate can be superimposed upon those obtained when small amounts of potassium oxalate were used without any identifying difference being discernible. The evidence indicates that for optimal Ac-globulin stability in human plasma the concentration range for these anticoagulants is low. No evidence is presented in this study to explain the mechanism of the difference between the results obtained with citrate and with oxalate.

*Silicone and Glass Surfaces.* Surface phenomena have long been appreciated as a factor involved in coagulation. By the use of silicone surfaces Jaques *et al.* (12) have shown that this nonwetting surface will extend the clotting time of native blood and plasma. Platelets do not agglutinate nor do they disintegrate so rapidly when the blood is in contact with silicone as compared to glass. In order to study the effect of surfaces upon prothrombin and Ac-globulin activity in stored plasma the following experiment was carried out. Whole blood was taken, centrifuged 15 minutes at 2000 r.p.m. and the plasma removed and stored, utilizing in one case siliconed surfaces throughout and in the other the usual glass.

The type of surface appears to have no effect upon prothrombin stability (fig. 4). However, the fall in activity by the original 2-stage prothrombin technic was retarded in the citrated plasma collected in silicone containers. The Ac-globulin level in citrated plasma fell more completely when in contact with the glass surface as compared to silicone (fig. 3). Oxalate appears to have altered the Ac-globulin so rapidly in this experiment that the possible effects of surface are not apparent.

Because most of the formed elements are removed at the intensity of centrif-

uation employed in this experiment and, also, considering the lack of a marked alteration in stability of the factors studied, it seems reasonable to conclude that

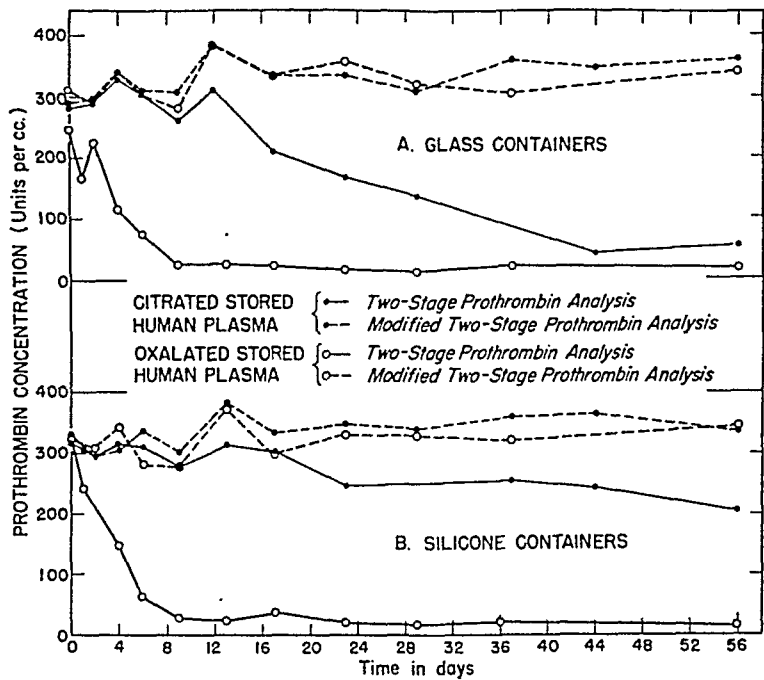


Fig. 3. PLASMA AC-GLOBULIN CONCENTRATION in glass (A) and siliconed (B) containers. Plasma obtained by standard methods.

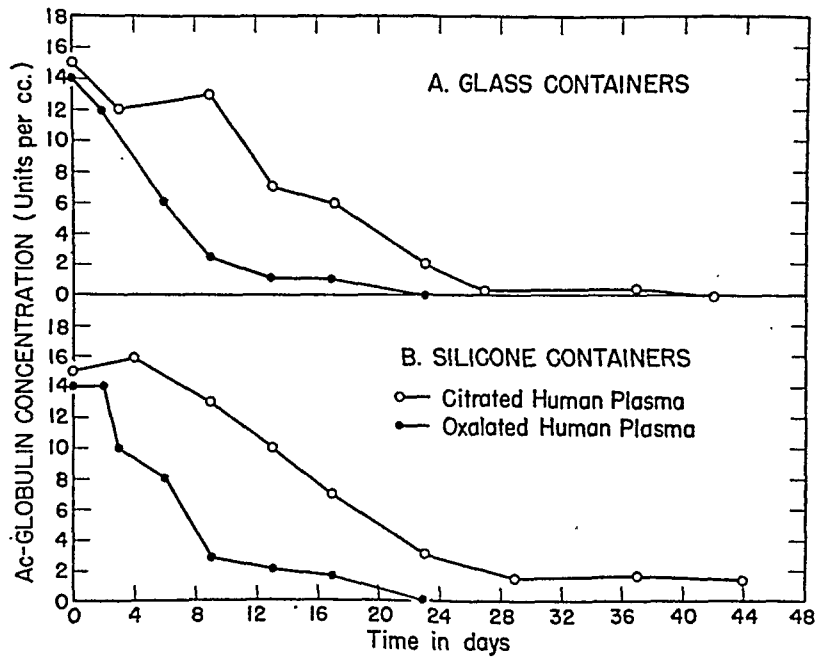


Fig. 4. PROTHROMBIN CHANGES in glass (A) and siliconed (B) containers on storage of human plasma. Determinations were carried out by the original and by the modified 2-stage procedure. Standard anticoagulant concentrations used.

there is no direct difference in the effect of a wetting and nonwetting surface upon the prothrombin or Ac-globulin stability of a stored plasma. However, presumably



in a glass container more platelet disintegration would occur than in a siliconed container before separation of the plasma from the cellular elements. A plasma in contact with glass when finally removed from above the precipitated cellular elements would contain a considerable amount of platelet breakdown products in comparison with the plasma taken from a siliconed container where platelet destruction was minimized. Thus the small difference in Ac-globulin stability within the two types of

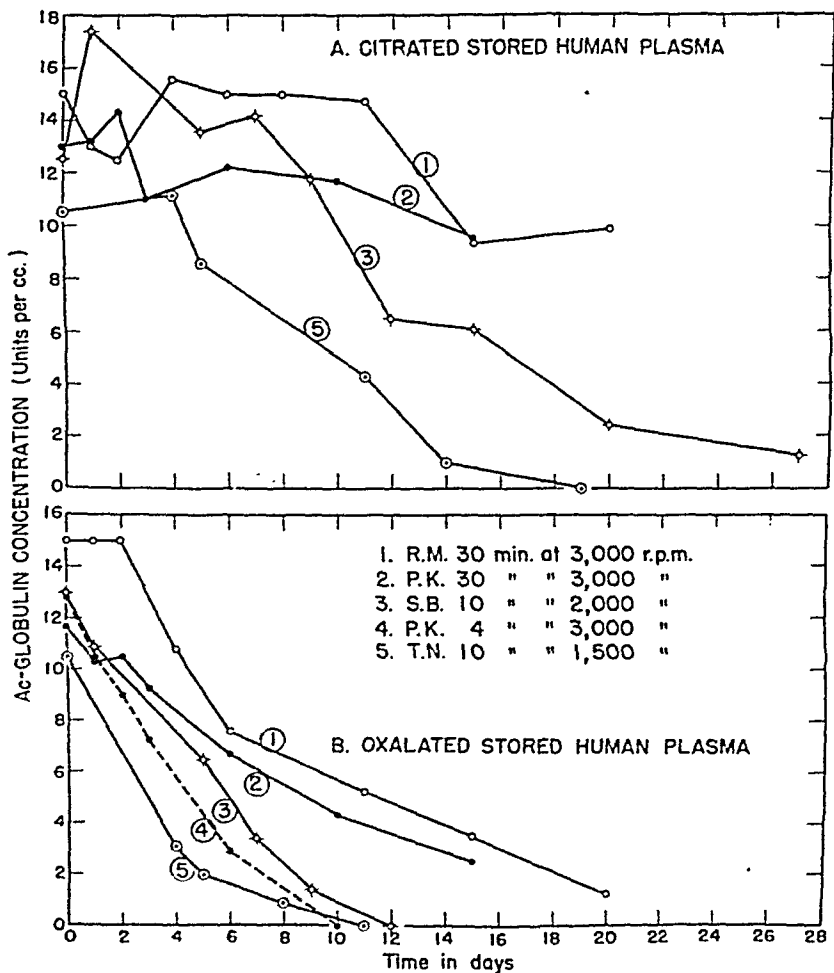


Fig. 5. INFLUENCE OF CENTRIFUGATION INTENSITY on Ac-globulin stability in stored citrated (A) and oxalated (B) human plasma. Plasmas 2 and 4 were obtained from the same drawing of whole blood. Whole blood was collected under standard conditions.

surfaces becomes of possible significance and would indicate that this might be due indirectly to the effect of these surfaces on the formed elements of the blood.

*Centrifugation.* In the course of these studies it was observed that the degree of centrifugation seemed to affect plasma Ac-globulin stability. Both oxalated and citrated human bloods were, therefore, subjected to rates and duration of centrifugation varying from 5 minutes at 1500 r.p.m. to 30 minutes at 3000. The plasma was then removed and stored. With increasing intensity of centrifugation and precipitation of more cellular elements the plasmas progressed from translucent to clear with respect to transmitted light. Platelets are the suspended elements involved as the minimal centrifugation removed most of the erythrocytes.

Ac-globulin stability decreases as the intensity of centrifugation is reduced (fig. 5). The evidence seems clearly to implicate platelets among those factors which influence the stability of Ac-globulin in stored plasma, the presence of platelets having an adverse effect upon maintenance of Ac-globulin activity.

*Platelet Factor.* To further study the effect of platelets upon the stabilizing ability of plasma an extract was prepared by differential centrifugal separation of the platelets, trituration and extraction with saline as described in an accompanying paper. To 3 volumes of fresh citrated human plasma which had undergone relatively hard centrifugation (20 minutes at 4500 r.p.m.) to remove the cellular elements was added 1 volume of the platelet extract or saline. The level of plasma Ac-globulin was followed under refrigerated storage conditions and the results charted in figure 6.

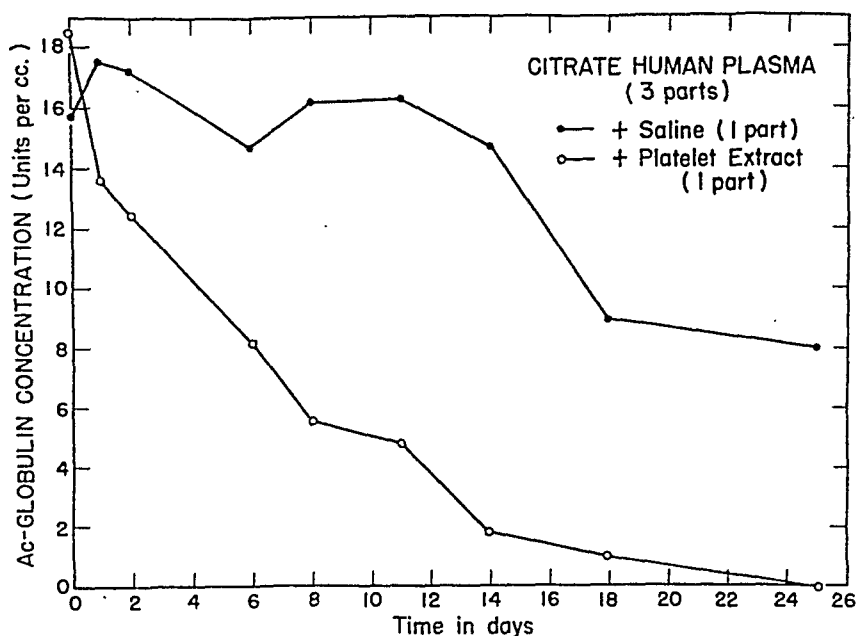


Fig. 6. EFFECT OF PLATELET EXTRACT ON Ac-globulin stability in stored human plasma decalcified by a standard sodium citrate concentration.

The initial high level in the plasma found one-half hour after mixing with platelet extract is to be expected because of the existence in platelets of a factor previously referred to which accelerates the prothrombin conversion rate and which is additive to the plasma Ac-globulin activity. By the end of 24 hours the inhibitory effect became manifest and the Ac-globulin titer was less in the platelet extract plasma than in the plasma with saline alone added. On continuation of storage the instability of Ac-globulin became more marked in the medium containing platelet products. This experiment has been repeated and confirmed in citrated and oxalated human plasmas. Furthermore, the quantity of platelet extract comparable to that obtainable from a physiological platelet concentration (13) causes a distinct decrease in Ac-globulin activity.

From these results it is evident that platelets contain a factor which decreases the stability of Ac-globulin in decalcified stored human plasma. This property may

be overshadowed initially by the activity of the accelerator liberated from the platelets. The inhibitory activity is manifest whether oxalate or citrate is used as anticoagulant.

*Further Studies on Prothrombin.* Table 2 summarizes the effects of the various storage conditions upon prothrombin. When storage was carried beyond two months the prothrombin level began to fall. Bacterial action may have been responsible for this alteration. Different concentrations of anticoagulants in the range studied seem to have no influence on the maintenance of prothrombin activity. Also the platelet factor appears not to be of importance in the stability of prothrombin in stored plasma. From this data and that recorded earlier the high stability of prothrombin

TABLE 2. EFFECTS OF VARIOUS STORAGE CONDITIONS UPON PLASMA PROTHROMBIN ACTIVITY AS MEASURED BY THE MODIFIED TWO-STAGE METHOD

A. SILICONE AND GLASS				B. ANTICOAGULANT CONCENTRATION		C. PLATELET EXTRACT
Plasma	Days stored			Concentration of anti-coagulant added to blood	Prothrombin after 30-day storage	Prothrombin after 39-day storage
	56	80	116			
	Prothrombin				U/cc.	U/cc.
	U/cc.	U/cc.	U/cc.			
Silicone				Sodium citrate		
Citrate	335	220	140	0.063 M	333	314 <sup>1</sup>
				0.125 M	333	
Oxalate	345	314	182	0.250 M	329	340 <sup>2</sup>
				0.500 M	322	
Glass				Potassium oxalate		
Citrate	341	270	181	0.05 M	333	320 <sup>3</sup>
Oxalate	330	285	174	0.10 M	300	341 <sup>4</sup>
				0.30 M	310	

<sup>1</sup> Sample prepared by mixing 3 parts citrated plasma with 1 part platelet extract before storage

<sup>2</sup> Sample prepared by mixing 3 parts oxalated plasma with 1 part platelet extract before storage

<sup>3</sup> Control: 3 parts citrated plasma mixed with 1 part saline before storage.

<sup>4</sup> Control: 3 parts oxalated plasma mixed with 1 part saline before storage.

in stored human plasma stands in contrast to the variable but more rapid decrease in activity shown by Ac-globulin.

## DISCUSSION

The conditions of collection and storage have an important relation to the stability of plasma coagulation factors. Determination of prothrombin and Ac-globulin concentrations by improved and specific methods show prothrombin to be quite stable, whereas Ac-globulin is much more sensitive to the conditions imposed upon the stored plasma. The presence of platelets or platelet extracts accelerates the rate of Ac-globulin inactivation. The effects of various surfaces and centrifugation intensities appear to exist because of their relation to the platelet content of plasmas. In addition, Ac-globulin is less stable in potassium oxalate than in sodium citrate at equimolar concentrations and less stable in both anticoagulants at higher concentrations.

In earlier reports on plasma or whole blood it is not always clear what the conditions of storage were. Also, the methods of analysis were not specific. The changes formerly attributed to prothrombin in stored human plasma were not due to alterations in the prothrombin itself, but were the result primarily of the change occurring in the separate plasma protein, Ac-globulin. Fibrinogen may or may not have been involved in results dependent upon the 1-stage method of analysis (14-17). Variations in fibrinogen do not effect results obtained by the 2-stage technic.

In general the changes measured by means of the 1-stage test indicated prothrombin to be unstable (18-22), but not all the reports agreed (23-25). Those groups of workers that have used both the 1-stage and the 2-stage methods of analysis report fairly high prothrombin stability by the 2-stage method. Ziegler, Osterberg and Hovig (26) feel that the correlation between the two methods is rather good. Lord and Pastore (27) on the other hand indicate considerable discrepancy. Both utilized bank blood. Warner, DeGowin and Seegers (28) found by the 2-stage technic that 50 per cent activity remained after three weeks in stored citrated plasma. A somewhat greater loss occurred by the 1-stage determinations.

In 1943 Quick (29) found that upon mixing stored human plasma and fresh dicumarol dog plasma an unexpectedly high prothrombin value was obtained by the 1-stage test. On the basis of this evidence he stated that upon storage of decalcified human plasma one factor in the thrombin-forming mechanism is labile while another remains stable. These he named, respectively, *component A* and *B* of prothrombin, and postulated that prothrombin is composed of these two essential and separable factors combined with calcium. Soon thereafter Seegers, Loomis and Vandenbelt (30) predicted a reinterpretation of Quick's data for, on the basis of extensive prothrombin purification work, they found no evidence for more than one component of prothrombin.

As early as 1939 Smith and his associates recognized variability in the prothrombin to thrombin conversion rate (31-33). This was encountered particularly in comparison of the times required for thrombin production in various species. These differences were attributed to a 'convertability factor of unknown nature' (34). In working with purified prothrombin Mellanby (35) and Seegers (36, 37) noted slow activation of prothrombin to thrombin.

More recently Owren (1, 2), Fantl and Nance (3, 38) and Ware, Guest and Seegers (4, 5) independently identified a substance in normal plasma which is necessary for the physiological transformation of prothrombin to thrombin. This factor was named accelerator globulin (Ac-globulin) by these last investigators because of its chemical and functional properties. It appears to be the same as Owren's *factor V* and the accelerator substance of Fantl and Nance. Honorato (39), referring to a plasmatic cofactor of thromboplastin to distinguish it from prothrombin, also appears to be dealing with this same substance. Further, from the evidence presented here and otherwise now known about plasma Ac-globulin it may be assumed that this factor is that with which Quick was primarily concerned when he first postulated the *component A* of prothrombin.

A modification of Quick's original concept and terminology was made by him in 1947 (40). *Component B* (prothrombin) was recognized as the compound or frac-

tion which is the true mother-substance of thrombin. The term *component A* was replaced by 'labile factor' as a name for the substance which decreases in activity in stored oxalated human plasma. Though not described as an accelerator 'labile factor' presumably applied to that plasma fraction referred to here as Ac-globulin. Actually Ac-globulin is stable in oxalated beef plasma (41) and in citrated human plasma when stored at refrigeration temperature. Only under certain conditions is this factor found to be unstable.

#### SUMMARY

The stability of prothrombin and of Ac-globulin has been studied in normal human plasma under various conditions of collection and of refrigerated storage. To accurately measure the level of prothrombin activity under storage conditions a modification of the original 2-stage method is required in which sufficient accelerator substance is provided for full conversion of prothrombin to thrombin. Prothrombin was found to be stable in oxalated or citrated stored human plasma.

Ac-globulin is sensitive to the conditions under which the experiment is carried out. At equal molar concentrations of anticoagulants the Ac-globulin is found to be less stable in oxalated than in citrated human plasma. At a high citrate concentration the Ac-globulin titer may fall as rapidly as in a lightly oxalated plasma. Silicone and glass surfaces exert no direct influence on Ac-globulin stability. However, the type of surface by means of its effect upon platelet stability indirectly is related to the control of plasma Ac-globulin stability. The degree to which platelets are separated from the plasma by varying the centrifugation intensity affects the amount of alteration in Ac-globulin on storage. Plasma is found to be a more stable medium when the platelet content is low. Addition of a platelet extract to decalcified human plasma decreases the plasma Ac-globulin stability.

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# CONCENTRATION OF PROTHROMBIN AND AC-GLOBULIN IN VARIOUS SPECIES<sup>1</sup>

ROBERT C. MURPHY AND WALTER H. SEEGER

*From the Department of Physiology, College of Medicine, Wayne University*

DETROIT, MICHIGAN

EVIDENCE has recently been reported indicating that plasma contains a factor which accelerates the rate of conversion of prothrombin to thrombin (1-5). Ware, Guest and Seegers (4) have termed this factor Ac-globulin since it is a globulin and since it accelerates the interaction of prothrombin and thromboplastin in the presence of calcium ions. Evidence has also been presented which indicates that Ac-globulin exists in two forms (6). The enzyme found in plasma (plasma Ac-globulin) is probably a proenzyme while that found in serum (serum Ac-globulin) is the active catalyst. It has been shown, using purified reagents, that thrombin is capable of activating the proenzyme (6, 7). As an explanation of the clotting reactions it has been postulated (6), therefore, that prothrombin interacts first with thromboplastin in the presence of calcium ions to form thrombin slowly. The thrombin in turn causes the change in activity of plasma Ac-globulin to serum Ac-globulin. The latter then intensifies the interaction of prothrombin and thromboplastin so that thrombin is formed rapidly. Fibrinogen is then converted to the fibrin clot through the action of thrombin.

Variations in the concentration of Ac-globulin could reasonably be expected to influence markedly the 1-stage prothrombin times (8) since this procedure measures the rate of thrombin formation (8). On the other hand, such an influence should be partially eliminated in the 2-stage method of prothrombin analysis of Warner, Brinkhous and Smith (9) since in this method the prothrombin is allowed to convert to thrombin before analysis is made. That some such factor is involved in these methods of assay is suggested by the marked discrepancies in the results reported by the two methods. Warner, Brinkhous and Smith (10) report the following prothrombin levels obtained by the 2-stage method of analysis: dog 100, rat 95, cat 91, rabbit 89, man 84, guinea pig 53, chicken 50, turtle 42. Quick (11), using the 1-stage method of analysis, found the following prothrombin relationships: dog 100, rabbit 100, cat 60, man 20 and cow 16. Warner, Brinkhous and Smith (10, 12) explained this discrepancy by postulating a 'convertability factor'. These workers noted that man and guinea pig prothrombin was converted slowly to thrombin in the 2-stage assay procedure, while dog and rabbit were 'rapid converters'. This offered an explanation for the relatively low prothrombin values for man and the high values of dog and rabbit reported by Quick (11). Other discrepancies between the 2-stage and the 1-stage methods have also been reported and explained on the basis of the convertability factor (13-15). If the original hypothesis concerning a convertability factor is correct one might expect a high Ac-globulin activity in dog and rabbit and lower activity in guinea pig and man. Partial purification and development of a quantitative method of analysis for Ac-globulin (16) has made possible a study of the concentration of this factor in these various species.

## EXPERIMENTAL

*Methods.* Blood was obtained from stock laboratory animals chosen at random. The blood was collected by arterial cannulation or by cardiac puncture and was

Received for publication June 14, 1948.

<sup>1</sup> Aided by a grant from the National Institute of Health.

mixed with 1.85 per cent potassium oxalate in the respective ratio of 7 to 1. A portion of the blood from each animal was permitted to clot spontaneously in clean dry test tubes. Cell free serum and plasma were obtained by centrifugation at 3000 r.p.m. for 30 minutes. All samples were kept at 5°C. until analyzed. Hematocrits were obtained on all specimens and all values reported are corrected for anticoagulant dilution.

Prothrombin analyses were performed by the 2-stage method of Warner, Brinkhous and Smith (9). Ac-globulin determinations were done according to the procedure described by Ware and Seegers (16). Purified prothrombin for Ac-globulin analyses was prepared according to methods of Ware and Seegers (17). To minimize variations in reagents all analyses were standardized to controls. These controls consisted of bovine plasma and bovine serum which were stored at -20°C.

*Plasma Ac-globulin Concentrations.* The results of the analyses of the plasmas of various species for Ac-globulin are shown in table 1. Significant differences in activity are seen. Of the mammals studied, man and guinea pig have the lowest Ac-globulin concentration while dog and rabbit have the highest, cow being intermediate. Some variation was noted in the type of prothrombin activation curves obtained with plasmas from the different species. This was especially true in the case of the rabbit plasma and may be a possible explanation of the variation in Ac-globulin concentration found with different samples from this species. Similar difficulty was also experienced in the interpretation of the activation curves produced by the rat, chicken, and turtle plasmas.

*Prothrombin Concentration.* Prothrombin analyses were also performed on the same plasmas and the results are shown in table 1. With the exception of the dog these results correlate fairly well with those obtained by Warner, Brinkhous and Smith (10). Repeated analyses have shown the prothrombin of the dog to range between 180 and 200 units per cc. while Warner has reported higher values for this species. The reason for this discrepancy is not clear.

Inasmuch as Ac-globulin has been shown to affect not only the rate of thrombin formation but also the thrombin yield (4, 5), it occurred to us that perhaps in some species a lack of Ac-globulin might be limiting the final thrombin yield and therefore giving too low a prothrombin value when measured by the 2-stage method. To test this possibility an optimum amount of Ac-globulin was supplied in the 2-stage method. For this purpose prothrombin-free bovine serum was supplied in the 2-stage method so that it was diluted 800 times in the final clotting mixture. The results are shown in table 1. It is evident that no outstanding difference was noted in the prothrombin values obtained by the 2-stage method of Warner, Brinkhous and Smith and by the modified method (18).

*Serum Ac-globulin.* Analyses of serum for Ac-globulin activity are shown in table 1. The data indicate that the values for the serum showed marked variation in activity from what might be expected when compared to homologous plasma values. Human and dog serums showed marked loss of Ac-globulin activity as compared to bovine. It was found that if human or dog blood was centrifuged for 10 minutes immediately after withdrawal, serum Ac-globulin activity of comparable degree to plasma could be demonstrated. Furthermore, it has been shown that on storage there is a rapid loss of Ac-globulin activity in these sera. Rabbit serum is



more like beef serum in that its Ac-globulin activity is relatively stable. In some instances as much as 50 per cent of the activity remained at the end of 24-hour storage at room temperature.

TABLE 1. CONCENTRATION OF PROTHROMBIN, PLASMA AC-GLOBULIN AND SERUM AC-GLOBULIN FOR VARIOUS SPECIES

SPECIES	PROTHROM- BIN (2-stage)	PROTHROM- BIN (2-stage, mod.)	PLASMA AC-GLOBU- LIN	SERUM AC-GLOBU- LIN <sup>1</sup>
	U/cc.	U/cc.	U/cc.	U/cc.
Cat	281	281	170	91.2
	253	253	123	60
	271	302	127	99
Dog	210	210	167	<1
	190	200	203	<1
	205	215	158	<1
Guinea pig	212	249	38	10.8
	203	212	40	<1
	210	214	31	<1
Rabbit	239	239	121	67
	249	249	142	233
	221	215	92	233
		265	310	
Ra	320	330	73	<1
	322	342	55	<1
	323	333		
Chicken	110	118	3.2	<1
	95	97	4.0	<1
	93	98	4.7	
Turtle <sup>2</sup>	68	70	3.0	<1
Human <sup>2</sup>	290	290-315	12-17	<1
Bovine <sup>2</sup>	250-285	250-285	120-140	70-90

<sup>1</sup> At room temperature, and 30 minutes after clotting in ordinary clean glassware.

<sup>2</sup> Pooled: 4 turtles; 12 humans; 15 cows.

*Comparison of Plasma Ac-globulin and Serum Ac-globulin.* The original investigations concerning the inactive form of plasma Ac-globulin and its change to active serum Ac-globulin as the result of the action of thrombin were made with media of bovine origin (7). The Ac-globulin activity in the sera of man and dog is so unstable it is possible to demonstrate the change in activity from plasma Ac-globulin to serum Ac-globulin only under specially controlled conditions. Therefore, the following experiment was performed in order to demonstrate the change of activity of plasma Ac-globulin to serum Ac-globulin in human blood.

Blood was obtained by venipuncture using syringes and glassware which had been treated with dri-film according to the technic of Jaques *et al.* (19). No anti-coagulant was used. The blood was centrifuged at 20,000 r.p.m. for one hour at 5°C. and the plasma, which was free of fibrin, was separated from the cells by the technic described by Patton, Ware and Seegers (20). The plasma was then analyzed for Ac-globulin activity. At room temperature coagulation of the plasma occurred slowly and the fibrin was removed. Analyses were then repeated on the serum under identical conditions. The curves in figure 1 represent the activation of purified bovine prothrombin by the Ac-globulin from the serum and plasma sources in the presence of thromboplastin and calcium ions. Curves A and B represent the activa-

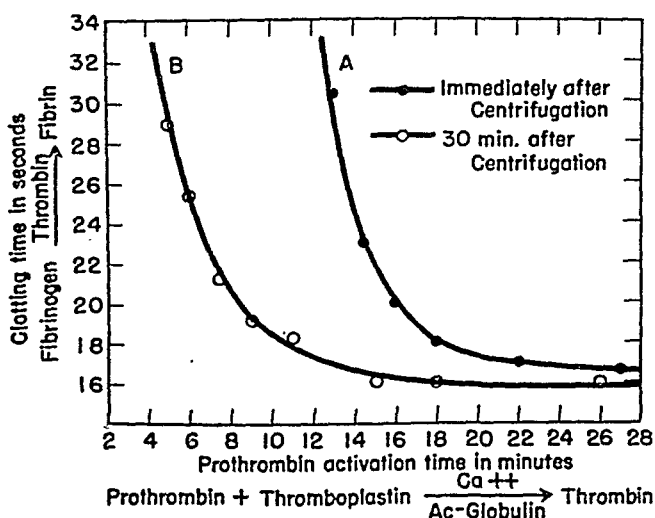


Fig. 1. ACTIVATION OF PURIFIED PROTHROMBIN SOLUTION with excess thromboplastin, optimum calcium ion concentration. Sources of Ac-globulin were a) human plasma collected with silicone technic diluted 1500 times and b) the same human plasma after slow spontaneous clotting and also diluted 1500 times.

tion of the prothrombin in the presence of Ac-globulin from human plasma before and after clotting had occurred. In each case the human serum and human plasma was diluted 1500 times in the final clotting mixture. It is evident, therefore, that a similar relationship exists between plasma and serum Ac-globulin of human origin as reported for bovine origin (6, 7). However, because of the instability of human serum Ac-globulin this relationship may be easily overlooked. If small amounts of thrombin, for example, about 2 to 5 U/cc., are added to human plasma the same change in the activity of Ac-globulin can be observed. A similar relationship between plasma Ac-globulin and serum Ac-globulin has been demonstrated with the plasmas of dog, rabbit and guinea pig.

#### DISCUSSION

The findings of plasma Ac-globulin concentrations of 150 to 200 U/cc. for dog, 12 to 17 U/cc. for man, and 30 to 40 U/cc. for the guinea pig offer adequate experimental support to the contention of Warner, Brinkhous and Smith (10, 12, 15) that the difference in prothrombin analyses in various species between the 1-stage and 2-stage methods is attributable to a factor which influences the rate of conversion

of prothrombin to thrombin. The prothrombin values of the mammals studied fell in the range of 200 to 300 u/cc. while the Ac-globulin concentration varied between 15 units for man to 350 units for rabbit. On the other hand, if the 1-stage method is utilized, the values for dog and rabbit are five times as great as for man. It would appear, therefore, that the concentration of Ac-globulin has a profound influence on the determination of prothrombin by the 1-stage method. Owren's description (3) of a syndrome characterized by a deficiency of a factor which we believe to be identical with Ac-globulin, resulting in an apparent prothrombin deficiency as measured by the 1-stage method, emphasizes the need for further consideration of the problem.

Evidence has been reported from other laboratories which substantiates the findings presented here. Owren (2) has reported the activity of Factor V to be greater in bovine and guinea pig plasma than in human plasma. Fantl and Nance (21) have shown that if human plasma is diluted with plasmas of various species from which the prothrombin has been removed by aluminum hydroxide or barium carbonate, shorter coagulation times result. Rabbit plasma causes the shortest coagulation time followed by dog and then guinea pig plasma.

In the bovine species both Ac-globulin and prothrombin values are relatively high (table 1). Curiously, Quick (11) reported low prothrombin values in this species. This discrepancy suggests that some factor other than Ac-globulin might be involved. The question of homologous reagents in species specificity cannot be ignored. The data available do not offer an adequate explanation for the discrepancy.

The use of a modified 2-stage method of prothrombin analysis has indicated that the original 2-stage method of Warner, Brinkhous and Smith is unaffected by increased amounts of Ac-globulin. It has been shown, however, that the 2-stage method also indicates apparent losses of prothrombin activity where exceptionally low Ac-globulin concentrations are involved (2, 4, 5). In such instances only the modified 2-stage technic gives reliable results. It is apparent, therefore, that some data obtained with the 2-stage technic may need to be reinterpreted. In work with purified prothrombin serious difficulty was encountered (17). Furthermore, Owren (3) showed that there is not sufficient Ac-globulin in parahemophilia to insure the full prothrombin titer. Experiments on storage plasma, currently being conducted in this laboratory, also show that a lack of Ac-globulin may effect the 2-stage method. It is not at once apparent why turtle plasma can be analyzed by either the original or modified procedure since its plasma contains only 3 u/cc. of Ac-globulin. It must, however, be realized that there is as yet no adequate information on the relative amounts of Ac-globulin which must be associated with prothrombin if the 2-stage method for prothrombin analysis is to be unaffected. In the turtle the ratio of prothrombin/Ac-globulin is 70/3 and in man about 300/12. The human species, therefore, has fewer Ac-globulin molecules for each prothrombin molecule than the turtle; in fact, fewer than any other species studied. On this basis it becomes apparent that a reduction of Ac-globulin below normal in man will markedly increase the ratio of prothrombin to Ac-globulin. This concept may be of considerable consequence. The low Ac-globulin activity of human plasma, and a high prothrombin/Ac-globulin ratio, suggests that a narrow margin of safety exists. If loss of Ac-globulin activity

occurs in diseased conditions, the effect on the coagulation mechanism may be serious. Furthermore, we anticipate that the ratio of prothrombin/Ac-globulin will be of fully as much significance as a knowledge of the absolute concentration of either of these factors. This idea was already implied in the work of Smith and his associates when they spoke of compensatory mechanisms for low prothrombin values (13, 14).

In most species, including man, serum Ac-globulin is not very stable. The cow and the rabbit are outstanding exceptions, but the reason is not clear. The ultimate cause of instability will probably be ascribed to the action of thrombin. Certainly it has been demonstrated that thrombin in sufficient concentration will destroy Ac-globulin (7). It does not seem necessary to postulate the existence of a special inhibitor of serum Ac-globulin, as has already been suggested by Owren (22).

#### SUMMARY

Analyses of plasmas of selected species indicate that the relative concentration of plasma Ac-globulin, in units per cc., is as follows: man 12 to 17, dog 150 to 200, cat 130 to 170, rabbit 150 to 300 and guinea pig 30 to 40. The conversion from plasma Ac-globulin to serum Ac-globulin has been shown to take place in human, dog, rabbit, guinea pig and cow plasma; however, the stability of serum Ac-globulin varies considerably in the species studied. In man and dog serum Ac-globulin is extremely labile while in bovine and rabbit serum the factor is much more stable. The reported differences in the concentrations of Ac-globulin in the various species offer at least a partial explanation for the reported discrepancies of prothrombin values obtained by the 1-stage and the 2-stage methods, the latter, when modified, giving the correct values.

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# PLATELET EXTRACTS, FIBRIN FORMATION AND INTERACTION OF PURIFIED PROTHROMBIN AND THROMBOPLASTIN<sup>1</sup>

ARNOLD G. WARE, JOHN L. FAHEY, AND WALTER H. SEEGER

*From the Department of Physiology, Wayne University College of Medicine*

DETROIT, MICHIGAN

IT HAS been generally accepted that blood platelets accelerate the blood coagulation process by supplying thromboplastin. Two groups of investigators, however, recently reported that platelets do not supply much thromboplastin but affect the coagulation process in other ways (1, 2). Mann, Hurn, and Magath (1) demonstrated that platelet extracts restore to normal the delayed prothrombin time of stored human plasma only when additional thromboplastin is also provided. They conclude that platelets supply very little thromboplastin but that they potentiate the action of tissue thromboplastin. Quick (2) reports that platelets accelerate the clotting reaction not by supplying thromboplastin but by furnishing a substance which changes an inactive form of thromboplastin to active thromboplastin.

The availability to our laboratory, in purified form, of a number of the principal factors which participate in the clotting reactions offered opportunity for elucidating further the exact rôle of platelets in blood coagulation. The results show that platelets contain only small amounts of thromboplastin. Two other substances are present. One is an accelerator which catalyzes the change of prothrombin to thrombin by thromboplastin and calcium ions. It acts like serum Ac-globulin. The second substance, like acacia (3) and some other colloids, hastens the action of thrombin on fibrinogen. We have found no previous reference to this factor and in this paper it is referred to as platelet factor 2.

## EXPERIMENTAL

*Preparation of Platelet Extracts.* Platelet extracts were prepared oxalated from bovine blood by fractional centrifugation. At the slaughter house 350 cc. of blood were collected from a stab wound and mixed immediately with 50 cc. of 1.85 per cent potassium oxalate solution. Within one hour after collection the blood was centrifuged at room temperature at 1500 r.p.m. until most of the red cells had settled. The plasma, which still contained many red cells, was withdrawn and centrifuged at 0° C. in an angle head centrifuge at 4500 r.p.m. for 20 minutes. The platelets and red cells packed at the bottom of the tube were then resuspended and thoroughly mixed with 30 cc. of saline. Red cells were mostly removed from this solution by light centrifugation at room temperature and the suspended platelets were removed by further centrifugation for 15 minutes at 3000 r.p.m. in conical tubes. The supernatant fluid was discarded and the platelet mass was carefully removed from the remaining red cells packed at the bottom of the tube. When these platelet concentrates were examined on slides prepared with Wrights' stain about 500 platelets were found for each erythrocyte.

Extracts of the platelet concentrates were prepared by thorough trituration in a mortar with a

Received for publication June 14, 1948.

<sup>1</sup> Aided by a grant from the United States Public Health Service.

small amount of saline. Additional saline was then added to bring the volume of the extract from 0.2 cc. of centrifuge packed platelets to 5 cc. The macerated platelet fragments were removed from the extract by centrifugation at 3000 r.p.m. for 15 minutes.

*Preparation of Prothrombin.* Prothrombin was prepared from bovine plasma by the most recently described methods (4). The last traces of Ac-globulin were removed by heating for two hours in water solution at 53° C.

*Preparation of Thromboplastin.* Two hundred cc. of crude thromboplastin prepared from bovine lung (5) were centrifuged 30 minutes at 45,000 r.p.m. in a Sharples Super Centrifuge. The precipitate was resuspended in 200 cc. saline, resedimented, resuspended in 25 cc. saline and centrifuged at 2000 r.p.m. for 5 minutes to remove gross particles. The thromboplastin remaining in the supernatant fluid is very active and free of Ac-globulin (4).

*Preparation of Fibrinogen.* The detailed procedure has already been described (6). Briefly, oxalated bovine plasma is frozen, allowed to thaw slowly, and the fibrinogen remaining insoluble at 2° C. is harvested by centrifugation, thorough washing and solution in saline. The product was used only when the total protein was at least 95 per cent coagulable with thrombin.

*Preparation of Thrombin.* Thrombin was prepared from purified prothrombin by a variety of procedures employed while studying methods for obtaining highly purified thrombin. This work is not complete as yet and a detailed description will very probably be offered for publication at a later date. All preparations used had a specific activity equal to or greater than those described by Seegers and McGinty (7).

*Analysis for Prothrombin.* Both the unmodified two-stage method of Warner, Brinkhous and Smith (5) and the modified procedure of Seegers and Ware (8) were used. The modification consists of the addition of serum Ac-globulin (diluted beef serum) in the first stage of the reaction to insure complete activation of prothrombin.

*Analysis for Thrombin.* The quantitative procedure described by Seegers and Smith (3) was followed.

## RESULTS

*Platelet Accelerator.* An accelerator of the first stage of coagulation was shown to be present in platelets by the same procedure used for demonstrating the presence of serum Ac-globulin in serum (9). When a dilute solution of purified prothrombin, standardized to contain 1.34 U/cc., is allowed to react with thromboplastin and calcium ions in the two-stage prothrombin procedure, the production of thrombin is quite slow and its eventual yield is comparatively low. If serum Ac-globulin is first added to the reaction mixture, the rate of thrombin production is increased in proportion to the quantity of Ac-globulin added and the yield of thrombin also increases. By increasing the Ac-globulin concentration stepwise, one obtains a series of prothrombin activation curves like those depicted on figure 1 (heavy solid lines). If plasma is used as a source of Ac-globulin one obtains a similar series of activation curves (10) but the shape of the curves is of a more rectangular nature (fig. 1, broken lines). The fundamental reason for the difference between plasma Ac-globulin and serum Ac-globulin resides in the fact that plasma Ac-globulin is the precursor of serum Ac-globulin (9) and must first be acted upon by thrombin in order to become the active accelerator.

When platelet extracts are examined by this procedure it becomes apparent that the extracts contain an accelerator of the first reaction of clotting. Furthermore it is present in an active form similar in that respect to serum Ac-globulin. In fact, the platelet extract can serve as a substitute for serum Ac-globulin, the kinetics of prothrombin activation being identical with either substance. This can be seen by

examination of figure 1 where two curves obtained with platelet accelerator show the same contours as those obtained with serum Ac-globulin. If a given solution of platelet accelerator is matched in accelerator activity with a solution of serum Ac-globulin both can be diluted serially and the accelerator activity in each will remain quantitatively identical. The serum Ac-globulin activation curves can, therefore,

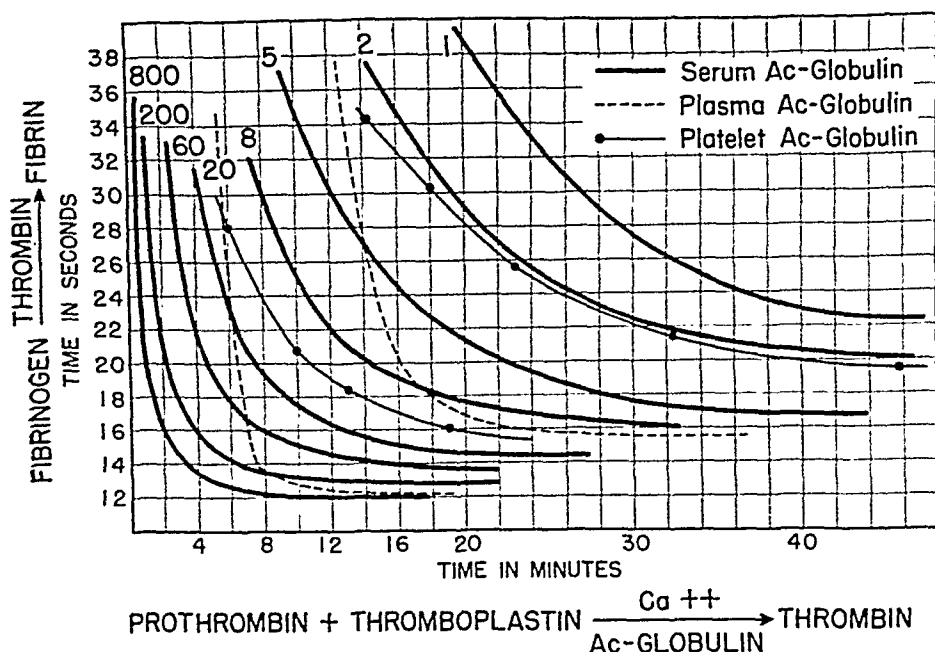


Fig. 1. ACCELERATION OF THROMBIN FORMATION by varying concentrations of serum Ac-globulin, plasma Ac-globulin, and platelet accelerator. The heavy continuous lines represent prothrombin activation curves obtained by incubating graded amounts of serum Ac-globulin with constant amounts of purified prothrombin (1.34 U/cc.), thromboplastin, and calcium ions for varying lengths of time (abscissa) under the conditions specified by the 2-stage procedure for prothrombin analysis (5). The thrombin formed in this reaction was measured by adding fibrinogen and recording the clotting time (ordinate). The prothrombin and thromboplastin were essentially free of accelerator. Concentrations of serum Ac-globulin (U/cc.  $\times$  1000) are represented by the large numbers at the tops of the curves.

The two broken lines represent prothrombin activation curves obtained by the same method of analysis but with two different concentrations of plasma Ac-globulin instead of serum Ac-globulin. The slopes of these curves are not the same as the curves for serum Ac-globulin because the inactive plasma Ac-globulin is being changed to active serum Ac-globulin during the period of incubation. The two light continuous lines represent prothrombin activation curves obtained by the same method of analysis but with two different concentrations of platelet accelerator. The slopes of these curves are identical with those for serum Ac-globulin. Both platelet and serum accelerators are active and are not proenzymes like plasma Ac-globulin.

be used for the quantitative determination of platelet accelerator activity. The assay procedure has accordingly been made identical to that already described for the quantitative determination of serum Ac-globulin (9), and, by definition, a unit of platelet accelerator is equivalent to a unit of serum Ac-globulin.

*Relative Amounts of Accelerator in Platelets and in Plasma.* A comparison of the activation of prothrombin in the presence of serum Ac-globulin and in the presence of platelet accelerator reveals that approximately one-twentieth of the total accelerator activity derived from bovine blood is from platelets. This comparison is

based on the assumptions that 100 cc. of bovine blood contains 0.5 cc. of centrifuge-packed platelets, and that the platelet accelerator is quantitatively extracted by the procedure outlined.

*Properties of Platelet Accelerator.* The physical and chemical properties of the accelerator present in the platelet extracts were investigated further. When heated at 53°C. for 10 minutes, 30 per cent of the activity was lost; when heated for 30 minutes, 87 per cent of the activity was lost. The accelerator remained fully active when the extracts were dialyzed against 0.9 per cent saline at room temperature. When a platelet extract was half saturated with ammonium sulfate, a white precipitate was formed. This precipitate, after centrifugation, solution in saline and dialysis to remove ammonium sulfate, contained 55 per cent of the original activity. This precipitate gave positive Biuret and Millon's tests. An aqueous solution of this precipitate was not coagulated by heat. From 70 to 80 per cent of the activity

TABLE 1. EFFECT OF PLATELET EXTRACT ON CLOTTING TIME OF A MIXTURE OF PURIFIED THROMBIN AND FIBRINOGEN

PLATELET EXTRACT	SALINE	THROMBIN SOLUTION	BUFFERED SALINE <sup>1</sup>	1% FIBRINOGEN	CLOTTING TIME
cc.	cc.	cc.	cc.	cc.	sec.
0	1	1	2	1	15.8
1	1	0	2		(clots in 5-10 min.)
1	0	1	2	1	11.0
0.5	0.5	1	2	1	12.7
0.25	0.75	1	2	1	14.1
0.125	0.875	1	2	1	15.1
0	1.5	0.5	2	1	30.0
1	0.5	0.5	2	1	19.7

All solutions were made in 0.9% sodium chloride. Temperature - 28°C.

<sup>1</sup> Made up so that the final clotting mixtures contained 1/10 its volume of imidazole buffer (11) and 0.15% calcium chloride (0.0136 M).

was sedimented by centrifugation for 45 minutes in a multispeed attachment rotor. The force was about 32,000 G.

*Platelet Factor 2.* This factor decreases the time required for thrombin to clot fibrinogen. Although this factor shows only moderate activity, failure to recognize its existence has probably resulted in faulty interpretations of experimental results.

The addition of a certain thrombin solution to purified fibrinogen caused a clot to form in 15.8 seconds (table 1). The addition of the same amount of thrombin plus platelet extract caused a clot to form in 11 seconds, even though the platelet extract alone did not clot the fibrinogen in 10 minutes. When a less concentrated thrombin solution, which caused clotting in 30 seconds, acted in the presence of platelet extract, the clotting time was reduced to 19.7 seconds. As an incidental observation it has been noted repeatedly that a mixture of thrombin and platelet extract produces a coagulum in about one minute. This coagulum is probably not fibrin even though it has much the same physical appearance. The coagulum can be



removed and the factor 2 effect can still be observed. Factor 2 can also be demonstrated with the use of oxalated bovine plasma (table 2).

*Properties of Platelet Factor 2.* Platelet extracts, prepared as described above, remained fully active when heated for 30 minutes at 53°C. All the activity also remained when the extract was dialyzed against saline for one hour. By half saturating an extract with ammonium sulfate, about half the activity could be demonstrated in the dissolved precipitate, after the latter solution had first been freed of ammonium sulfate by dialysis. All the activity remained in the supernatant solution after centrifugation at 32,000 G. for 45 minutes.

*Thromboplastin in Platelet Extracts.* In order to obtain an estimate of the thromboplastic activity of platelet extracts they were allowed to react with purified prothrombin both in the presence and in the absence of Ac-globulin. The pH was adjusted to 7.2 by addition of imidazole buffer (11). Calcium chloride was supplied at a concentration of 0.15 per cent (0.0136 M) in the final mixtures. When platelet extracts were incubated at 28°C. with sufficient prothrombin to give a final concen-

TABLE 2. EFFECT OF PLATELET EXTRACT ON CLOTTING TIME OF A MIXTURE OF PURIFIED THROMBIN AND OXALATED BOVINE PLASMA

PLATELET EXTRACT <sup>1</sup>	OXALATED BOVINE PLASMA	SALINE	THROMBIN <sup>1</sup>	CLOTTING TIME
				sec.
0	2 parts	1 part	1 part	25
1 part	2 parts	0	1 part	1.76
0	2 parts	1 $\frac{2}{3}$ part	$\frac{1}{3}$ part	105
1 part	2 parts	$\frac{2}{3}$ part	$\frac{1}{3}$ part	45
1 part	2 parts	1 part	0	no clot

<sup>1</sup> Made up in 0.9% sodium chloride solutions.

tration of 1.34 U/cc., no detectable amount of thrombin was formed in periods up to one hour. Neither did the addition of purified serum Ac-globulin to the mixture of prothrombin and platelet extract result in thrombin formation in a one-hour period. Addition of tissue thromboplastin to the mixtures of prothrombin and platelet extracts caused a rapid production and maximum yield of thrombin in three to five minutes.

When the prothrombin concentration was increased to 330 U/cc., approximately physiological concentration, the addition of platelet extracts caused a slow but definite production of thrombin. A 20 per cent yield of thrombin developed in a period of 2 hours and after 24 hours the titer was not much higher. These experiments indicate that the platelet extracts contain only a small amount of thromboplastin, if any. The experiments do not indicate whether the platelet accelerator can activate some prothrombin. This possibility seems remote.

#### DISCUSSION

In agreement with recent reports of others (1, 2) which were based on clotting times of mixtures of plasmas taken from various individuals, our results obtained

with purified prothrombin indicate that platelets contain only a small amount of thromboplastin. The relatively large amount of accelerator present in platelets was probably mistaken by early investigators for thromboplastin. Feissly (12) mentions the presence of two thromboplastic substances in platelets, one of which is heat stable and the other heat labile. His thermolabile platelet thromboplastin is probably the platelet accelerator described in this paper and should properly be regarded as an accelerator rather than as thromboplastin.

Platelet accelerator and serum Ac-globulin (9) appear to be similar with respect to their acceleration of prothrombin activation, their precipitation by half saturation with ammonium sulfate, and their destruction by heating at 53°C. The two accelerators differ somewhat, however, in their thermo-stabilities at 53°C. (table 3), serum Ac-globulin being destroyed more rapidly. A marked difference between the two substances lies in the fact that platelet accelerator is mostly sedimented at high centrifugal speeds, whereas serum Ac-globulin, in the form of bovine serum, is not sedimented under these conditions. Therefore, it appears that the two accelerators are entirely different proteins.

TABLE 3. STABILITY OF PLATELET EXTRACTS AND OF SERUM AC-GLOBULIN AT 53°C.

TIME	Platelet Extracts		Serum Ac-Globulin
	Factor 1 %	Factor 2 %	%
min.			
0	100	100	100
10	70	100	
15			7
30	13	100	0

Platelet factor 2 decreases the time of clot formation when present in mixtures of thrombin and fibrinogen. This property of platelet extracts diminishes readily on dilution so that it could not be expected to affect the rates of clot formation in highly diluted plasmas. However, where the plasma is used in high concentrations it is feasible that enough platelet factor 2 could be present to decrease clotting times appreciably.

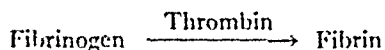
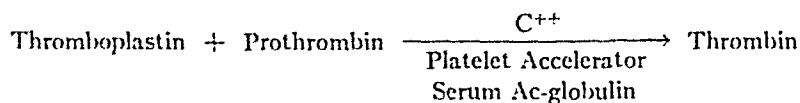
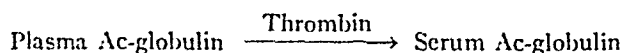
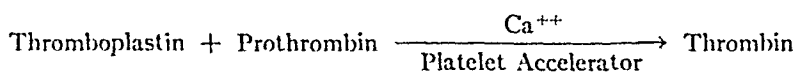
Platelet factor 2 may be associated in some way with the substance in these platelet extracts which clots in the presence of thrombin. This substance is very similar in appearance to fibrin. However, its precursor is probably not fibrinogen because it appears to be unaffected by heating at 53°C. for 30 minutes. In addition, it is not coagulated and remains clottable with thrombin even after being heated. Fibrinogen is completely denatured under these conditions. It remains to be seen whether these ideas can be developed.

Before attempting to integrate the information on platelet extracts with recent observations on prothrombin activation it is necessary to review certain pertinent facts. a) It has been shown that thrombin can be derived from purified prothrombin even in the absence of thromboplastin, Ac-globulin or calcium ions (4). This indicates that prothrombin, by itself, contains all the necessary structural material for the formation of the thrombin molecule, and further that thromboplastin, Ac-globulin and calcium ions act merely as catalysts of thrombin formation. It appears

that the only way to escape this conclusion is to postulate that the thrombin formed in the presence of thromboplastin, Ac-globulin, and calcium ions is different from that derived from prothrombin in their absence. *b*) Thromboplastin and calcium ions, in the absence of Ac-globulin, activate purified prothrombin slowly with a loss in total yield of thrombin (4). *c*) Plasma Ac-globulin is activated by small amounts of thrombin (9).

The experiments reported in this paper demonstrate that platelets contain an active accelerator and also small amounts of thromboplastin. These platelet constituents appear to be present in sufficient amounts to catalyze the formation of enough thrombin to change the inactive Ac-globulin of the plasma to the active form. In this way the platelets appear to retain their traditional rôle of trigger mechanism. This was recently again demonstrated very convincingly by the work of Brinkhous (13). More data are required for the rigid formulation of the exact rôle of thromboplastin of platelet origin and of the relative effects of the various amounts of prothrombin, Ac-globulin, calcium and thromboplastin on thrombin formation. Probably, under physiological conditions, thromboplastin of tissue origin is the chief source of this substance.

Although a number of uncertainties remain, it nevertheless seems appropriate to attempt to harmonize known facts as follows: thromboplastin of platelet origin, tissue origin, or both, according to the circumstances of the wound or the drawing of the blood, acts on plasma prothrombin to form a small amount of thrombin; platelet accelerator serves to catalyze the initial stages of this reaction; plasma Ac-globulin is activated by the first small amount of thrombin to serum Ac-globulin. The latter then becomes the main accelerator; and, finally, thrombin is produced rapidly and a clot forms. This correlation of events in clotting is in harmony with those made previously (9, 14, 15) on the basis of plasma constituents alone. The equations may be represented as follows:



As the minor source of accelerator, platelets serve the primary function of beginning the events of clotting. Serum Ac-globulin furnishes the 'follow through'. A deficiency of either can thus cause a bleeding diathesis but neither of the two substances is absolutely necessary for thrombin production.

It has been pointed out previously that no one-stage method for prothrombin analysis can distinguish between the concentration of prothrombin and its activation rate (8, 16). The one-stage methods also cannot account for variations due to fibrinogen reactivity (17) and for variables due to the plasma medium itself from one species to another (3). Two further variables must now be considered; namely,

a) platelet accelerator and b) platelet factor 2, both of which may affect the rates of clot formation.

#### SUMMARY

With the use of purified clotting agents it has been shown that bovine platelet extracts contain an accelerator of prothrombin activation. They contain only a small amount of thromboplastin. The accelerator is apparently present in platelets in the active form and acts in a similar manner to serum Ac-globulin. The platelet accelerator is apparently a protein. It is destroyed by heating at 53°C., is non-dialyzable and is precipitated by half saturation with ammonium sulfate. It is sharply distinguished from Ac-globulin by being mostly sedimented by centrifugation at 32,000 G.

Bovine platelets also contain a substance which hastens the second stage of clotting. This substance is non-dialyzable, stable to heat at 53°C. for 30 minutes, precipitated by half-saturation with ammonium sulfate, and is not sedimentable by centrifugation at 32,000 G. Platelet extracts shorten considerably the clotting time of bovine plasma.

It has been postulated that platelets aid in the initial formation of thrombin primarily by catalyzing the interaction of prothrombin and thromboplastin. This thrombin then activates the inert plasma Ac-globulin to its active counterpart, serum Ac-globulin, which acts as the principal accelerator of the first stage of clotting.

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# ABILITY OF THE STOMACH TO PRODUCE ELECTRICAL ENERGY

WARREN S. REHM AND LOWELL E. HOKIN

*From the Department of Physiology, University of Louisville, School of Medicine*

LOUISVILLE, KENTUCKY

**A**N ATTEMPT has been made in previously published work to throw further light on the intimate mechanism of gastric secretion by studies on the electrophysiology of the stomach. Specifically, the experiments were designed to test the hypothesis that electrical energy provided the energy necessary for the production of the osmotic work involved in the formation of the HCl of gastric secretion. Implicit in this hypothesis is the assumption that the mucosa of the stomach can produce sufficient electrical energy for the required amount of osmotic work.

In a former paper (1) an attempt was made to determine the ability of the stomach to produce electrical energy by placing low resistance, non-polarizable electrodes on opposite sides of the stomach wall and shunting these through a low external resistance. These findings can be illustrated by reference to the equivalent circuit of figure 1A where  $E_s$  represents the electromotive force of the stomach,  $R_s$  the resistance of the stomach wall,  $R_e$  the resistance of the electrodes applied to opposite sides of the stomach wall,  $R_x$  a fixed resistance of one ohm, and S a switch for closing the circuit. The potential difference (P.D.) was measured across the stomach wall with a potentiometer with switch S open. This switch was then closed and the electrical current in the external circuit was determined by measuring the IR drop across the one-ohm resistor. It was found that the current in the external circuit remained relatively constant with respect to time. Evidence was presented indicating that the total IR drop through the circuit was essentially equal to the open circuit P.D. across the stomach, and that the electromotive force, therefore, did not decrease with time. The average electrical energy production, under these conditions, was found to be 8.9 microwatts per cm.<sup>2</sup>, which is only a small fraction of the amount of energy needed for the production of the HCl of the gastric secretion. It was pointed out, however, that this method probably does not measure the total ability of the electromotive forces of the stomach to produce electrical energy and that the total production of electrical energy might be much greater than that found with this method. For example, if there are shunts inside the stomach, this method would underestimate the stomach's ability to produce electrical energy. As will be seen in the following analysis, the problem of determining how much electrical energy the stomach can produce is an extraordinarily difficult one. There is one approach, however, that might be expected to throw further light on the problem. This approach is based on the finding that the magnitude of the electromotive force giving rise to the P.D. across the stomach wall apparently does not decrease with time during the flow of current under the conditions in the above described experiments. Since the electromotive force does not apparently decrease with time, it is reasonable to believe that it could produce more electrical energy than the amounts obtained in the above experiments. The question arises as to how much more electrical energy the electromotive force of the stomach, giving rise to the P.D. across the stomach wall, can produce. The present paper is primarily concerned with an attempt to obtain an answer to this question.

The essential principle of the method used in the present paper can be illustrated by reference to the circuit in figure 1B. This circuit is essentially the same as the circuit of figure 1A except that an external battery,  $E_x$ , is placed in series with the stomach. The electrical energy produced in the

Received for publication May 7, 1948.

circuit would be equal to  $(E_x + E_s)I$  and that portion produced by the stomach equal to  $E_s I$ . The maximum ability of  $E_s$  to produce electrical energy (assuming no shunts in the stomach) could be determined by increasing the magnitude of  $E_x$  so as to increase the current  $I$  until the product of  $E_s$  and  $I$  reaches a maximum. The most difficult aspect of this problem is the determination of  $E_s$  during the passage of current across the stomach. It is conceivable that with this method the electrical energy production of  $E_s$  would be greater than the maximum electrical energy that could be obtained from  $E_s$ , if it were possible to connect a shunt of very low resistance directly to the 'terminals' of  $E_s$ . This possibility would depend on the relationship between the internal resistance of  $E_s$  and its ability to produce electrical energy when connected in series with an external electromotive force. It should be pointed out that the internal resistance of  $E_s$  is less than the total resistance across the stomach wall since it has been shown (13) that  $E_s$  originates somewhere between the submucosa and mucosal surface (probably nearer the mucosal than submucosal surface).

The magnitude of  $E_s$  could theoretically be determined during a period of current flow through the stomach, by measuring the P.D. across the stomach during this period. From the laws of electrical networks it follows that:

$$E_s = E_m - RI \quad 1$$

where  $E_m$  is the P.D. measured across the stomach wall,  $R$  the resistance of the stomach wall, and  $I$  the magnitude of the current passing through the stomach. However, accurate calculations of  $E_s$  would depend on  $R$  remaining constant, and, since changes in  $E_m$  could be due to changes in  $R$ , this method would not yield reliable values for  $E_s$ .

The method used to determine  $E_s$  in the experiments described below was one in which, during a period of current flow, the circuit was momentarily broken at definite intervals and the P.D. across the stomach (open circuit voltage) measured with a string galvanometer. If the actual circuit of the stomach is that represented in figure 1A, i.e., a resistance and an electromotive force in series (assuming for the present that there is no electrical capacity of the stomach), the P.D. measured immediately after the break of the circuit would be equal to the electromotive force immediately before the circuit was broken. The electrical energy production by the stomach during current flow would then be equal to the product of this P.D. and the current in the external circuit during the flow of current. Since the actual circuit of the stomach is not known, the question arises as to whether the product of this P.D. and the current would be an accurate measure of the electrical energy production of the stomach if the actual circuit of the stomach was different from that assumed above. For example, if the actual circuit was one (fig. 1D) in which there was a shunt ( $R_2$  of fig. 1D) across the electromotive force, would the above method be valid?

Application of the classical laws of electrical networks to the circuit represented in figure 1D reveals that

$$W = \frac{E_1 R_2}{R_1 + R_2} I_x + \frac{E_1^2}{R_1 + R_2} \quad 2$$

In this equation  $W$  represents the electrical energy production of  $E_1$  in watts when  $E_1$  is in volts, the resistances  $R_1$  and  $R_2$  in ohms, and the current in the external circuit  $I_x$  in amperes. It can easily be shown that the open circuit P.D. from  $S_2$  to  $M_2$  is

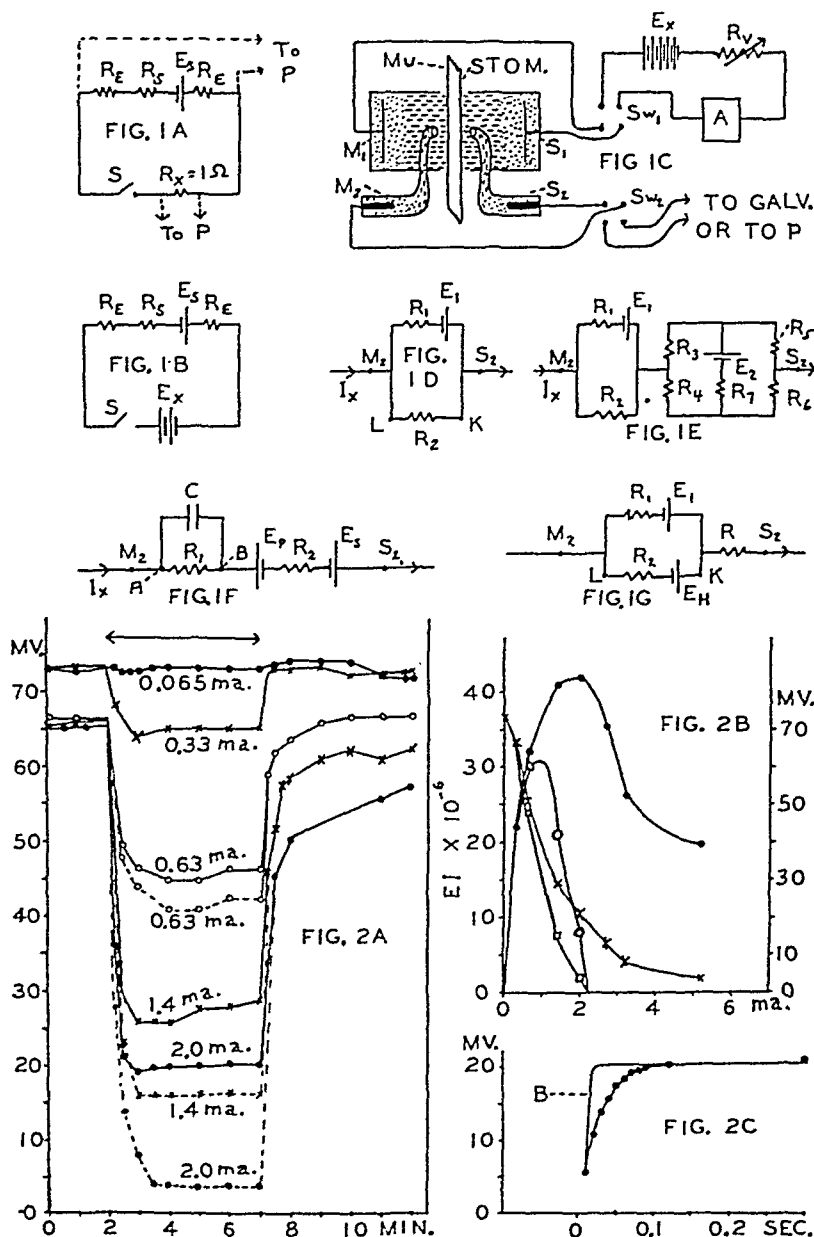


Fig. 1A. IN THIS CIRCUIT  $E_s$  represents the electromotive force of the stomach;  $R_s$ , the resistance across the stomach wall;  $R_E$ , the resistance of nonpolarizable isoelectric electrodes applied to the serosal and mucosal surfaces of the stomach;  $R_x$ , a resistance of one ohm;  $S$ , a switch and  $P$  a potentiometer.

Fig. 1B.  $E_x$  REPRESENTS A BATTERY. The other symbols have the same meaning as those of fig. 1A.

Fig. 1C. MU REPRESENTS THE MUCOSAL SIDE OF THE STOMACH,  $S_1$  and  $M_1$  electrodes placed opposite each other on the mucosal and serosal surfaces,  $S_2$  and  $M_2$  non-polarizable Zn-Zn acetate agar electrodes. The portions of the electrodes  $S_1$  and  $M_1$  in contact with the stomach were composed of 0.6% NaCl agar. Saturated KCl agar was present in electrodes  $S_2$  and  $M_2$  to make contact with the saline agar.  $Sw_1$  and  $Sw_2$  represent single throw, double pole switches;  $E_x$ , several lead storage batteries in series.  $R_v$ , a variable resistance;  $A$ , a milliammeter;  $Galv.$ , a string galvanometer and  $P$ , a potentiometer.

Figs. 1D and 1E. See text.

Fig. 1F. AN EQUIVALENT CIRCUIT OF THE STOMACH.  $C$  represents a static capacity in parallel with a resistance  $R_1$ ,  $E_p$  a polarization electromotive force resulting from the flow of current,  $E_s$  the electromotive force of the stomach giving rise to the P.D. across the stomach wall in the resting condition.

equal to  $\frac{E_1 R_2}{R_1 + R_2}$ , and that  $\frac{E_1^2}{R_1 + R_2}$  is equal to the electrical energy production by  $E_1$  after the external circuit has been broken ( $I_x$  equals zero). It is true (as a corollary of Thévenin's theorem, see Bush, 2) that for any circuit (assuming no capacity nor inductance) that the total electrical energy output of the electromotive forces of the circuit, during the flow of an external current through the circuit, is equal to the product of the P.D. measured immediately after breaking the circuit and the current in the external circuit before the break of the circuit, plus the electrical energy production in the circuit immediately after the circuit is broken. Therefore, on the basis of the above considerations, the conclusion is warranted that the method used in the present work will not over-estimate the ability of the stomach to produce electrical energy, and will under-estimate it if there are shunts across the electromotive forces by an amount equal to the electrical energy production inside the stomach immediately after breaking the circuit.

An implication of the above analysis should be pointed out, and that is the possibility (and not an unlikely possibility considering the histology of the stomach) that there are electromotive forces oriented in such a way that their electrical energy output would not be influenced (or only to a small extent) by the electrical current sent through the stomach. A circuit illustrating this possibility is shown in figure 1E. In this circuit it is assumed that the resistances  $R_3$ ,  $R_4$ ,  $R_5$ , and  $R_6$  are equal to each other and, therefore, the electromotive force  $E_2$  would not contribute to the P.D. between  $S_2$  and  $M_2$ . The passage of current through the circuit from an external battery would not change the electrical energy output of  $E_2$ . These conclusions can be easily verified by the application of the classical laws of networks to this circuit. Therefore, the method used in the present work could only be expected at best to determine the ability of those electromotive forces to produce electrical energy which are oriented in such a way as to contribute to the P.D. across the stomach.

For the sake of simplicity it was assumed in the foregoing analysis that the stomach did not possess an electrical capacity. Since the stomach undoubtedly does possess a capacity, the P.D. across the stomach following the break of the circuit would theoretically be a function of both the electromotive force and the discharge of the capacity. The electrical energy production, on the basis of the foregoing

Fig. 1G. See text.

Fig. 2A. EFFECT OF CURRENT FLOW ON P.D. ACROSS STOMACH. Period of current flow indicated at top of figure. Current density given in ma. per cm.<sup>2</sup> P.D. determined during period of current flow by momentarily breaking circuit and measuring P.D. with string galvanometer. Solid lines represent P.D. determined by ignoring rapidly rising phase in string galvanometer records. Broken lines represent P.D. determined by using initial values of galvanometer records.

Fig. 2B. ELECTRICAL ENERGY PRODUCTION of electromotive force of stomach in microwatts per cm.<sup>2</sup> vs. current density in milliamperes. Solid dots represent electrical energy production calculated from P. D. obtained by ignoring rapidly rising phase of galvanometer records. Open circles represent electrical energy output calculated from P.D. obtained by using initial values of galvanometer records. Crosses represent P.D. vs. current in which P.D. was determined by ignoring rapidly rising phase of records; squares represent P.D. vs. current in which P.D. represents initial readings of records.

Fig. 2C. P.D. vs. TIME, obtained from typical galvanometer record after interruption of current. Solid dots represent actual values obtained from the record; line through solid dots plotted from equation 4 of text. Line B represents plot of equation 4 in which  $R_1$  was assumed to be equal to the total resistance of the stomach.



analysis, would be at least equal to the P.D. across the stomach, due to the electromotive force times the current. In order to obtain the value for the P.D. due to the electromotive force, the measured P.D. would theoretically have to be corrected for the effect of the discharge of the capacity. This can be illustrated by reference to the equivalent circuit shown in figure 1F, where  $C$  represents a static capacity. Immediately after the break of the circuit the P.D. between  $S_2$  and  $M_2$  would be equal to  $E_s$  (assume  $E_p$  in this circuit to be zero for the time being) minus the P.D. between points A and B due to the discharge of condenser  $C$ . On the basis of measurements of the capacity of a wide variety of living tissues (3-6, 8) it has been found that the capacity of tissues in general is in the neighborhood of one microfarad per  $\text{cm}^2$ . Assuming the stomach possesses a capacity of approximately this magnitude and that  $R_1$  of figure 1F is equal to the total resistance of the stomach, it can be shown (see formula below) that the P.D. between points A and B of figure 1F, due to the discharge of  $C$ , would decrease to less than one mv. within a few milliseconds. The technique used in the following experiments was one in which the P.D. was measured with a string galvanometer at a relatively low camera speed so that the initial values of the galvanometer records would not be expected to be a function of the discharge of the capacity. However, it was found that with relatively high current densities an initial rapidly rising phase of the P.D. was present in the galvanometer records. The possibility of this phase being due to the discharge of the capacity is discussed below.

#### METHODS

A portion of the opened stomach of dogs anesthetized with sodium amytal (70 to 90 mg./kg. subcutaneously) was placed between two pairs of electrodes. One pair of the electrodes  $S_1$  and  $M_1$  (fig. 1C) was connected in series with lead storage batteries  $E_x$ , a variable resistance  $R_r$ , a milliammeter  $A$ , and a double pole, single throw switch  $Sw_1$ . The area of electrodes  $S_1$  and  $M_1$  in contact with the stomach was  $11 \text{ cm}^2$ . The other pair of electrodes  $S_2$  and  $M_2$  was connected to either a Hindle string galvanometer (without amplification) or a type K Leeds and Northrup potentiometer, via the double pole, single throw switch  $Sw_2$ . The electrode  $S_1$  was placed against the serosal surface and electrode  $M_1$  was placed opposite electrode  $S_1$  on the mucosal surface. A variable resistance and a calibrating voltage (10 mv. steps, not shown in fig. 1C) were placed in the string galvanometer circuit. The string galvanometer was calibrated before and after each experiment with switch  $Sw_2$  closed and switch  $Sw_1$  open. Changes in the resistance of the circuit, comparable to the possible changes in the resistance of the stomach, produced no appreciable change in the galvanometer readings. In the majority of the experiments a portion of the stomach wall was placed in a lucite chamber also containing two pairs of electrodes which will be referred to in the same way as those in figure 1C. (See former paper, 9, for a detailed description of this chamber.)

The handles of switches  $Sw_1$  and  $Sw_2$  were fastened to a lever so that movement of the lever opened one of the switches and closed the other one. In this way current flow through the stomach could be interrupted and the string galvanometer circuit connected to the electrodes  $S_2$  and  $M_2$ . In order to determine the total time elapsing

from the opening of switch  $Sw_1$  to the first reading on the string galvanometer, both switches  $Sw_1$  and  $Sw_2$  were connected to the same voltage source and the lever connecting the switches was thrown and it was found that the break in the string galvanometer record was of about 5 milliseconds' duration.

The following experiments were performed on non-secreting stomachs unless otherwise specified.

### RESULTS

Figure 2 represents the results of typical experiments in which currents of various magnitudes were sent through the stomach for five-minute periods. The positive pole of the external battery ( $E_x$  of fig. 1C) was connected to the mucosal current sending electrode ( $M_1$  of fig. 1C). The P.D. was measured before and after the current

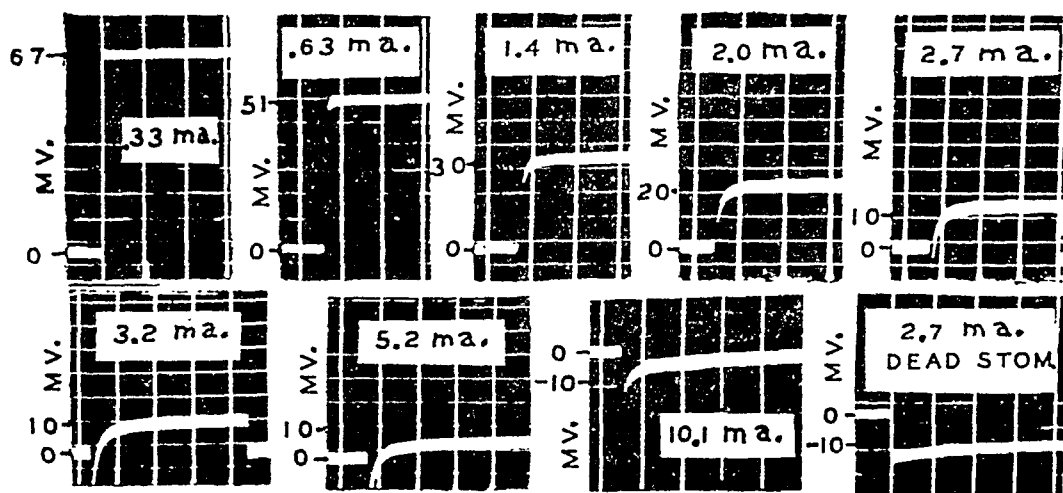


Fig. 3. STRING GALVANOMETER RECORDS OF P.D. across stomach following current flow of five minutes' duration. Numbers at top of each record represent current density in ma. per cm.<sup>2</sup>. Time intervals between vertical lines equal to 0.20 second. Numbers at left of records represent P.D. in mv. Record in lower right corner represents the effect of current flow on a dead stomach.

sending period with the potentiometer. Current of a given magnitude was sent through the stomach and the circuit was broken at intervals, and the open circuit P.D. measured during these intervals with the string galvanometer. With the higher current densities (fig. 3) the string galvanometer record showed an initial rapidly rising phase. On the assumption that this rapidly rising phase is not due to a rapid change in  $E_s$  of figure 1F (or  $E_1$  of fig. 1D), but to the decay of a back electromotive force ( $E_p$  of fig. 1F) or to the discharge of the capacity of the stomach, the P.D. used for the calculations of the electrical energy was determined by extrapolating the plateau portion of the curves to zero time (fig. 2C). In figure 2A the solid lines represent typical experiments in which the P.D. was determined in this way. It can be seen that with increasing current densities the magnitude of this P.D. is decreased. It can also be seen that for a given current density the value of this P.D. reached a relatively constant level within the first two minutes.

The P.D. for the calculation of the electrical energy output of the stomach was also determined on the assumption that the rapidly rising phase of the galvanometer

records is due to a rapid change in  $E_s$  (or  $E_1$  of fig. 1C). With this assumption the initial readings of the galvanometer records were used in calculating the electrical energy output of the stomach. In figure 2A the dotted lines represent the change of the magnitude of the P.D. determined in this way with time. It can be seen that the magnitude of the P.D. determined in this way also reaches a relatively constant value within approximately two minutes.

Figure 3 represents the string galvanometer records in a typical series of experiments after the current had been flowing for five-minute periods. The records obtained at the end of two, three, and four minutes were essentially the same as those shown in the figure. It can be seen that with current densities of 0.63 ma. per cm.<sup>2</sup> or less there was practically no rapidly rising phase of the curve, while a rapidly rising

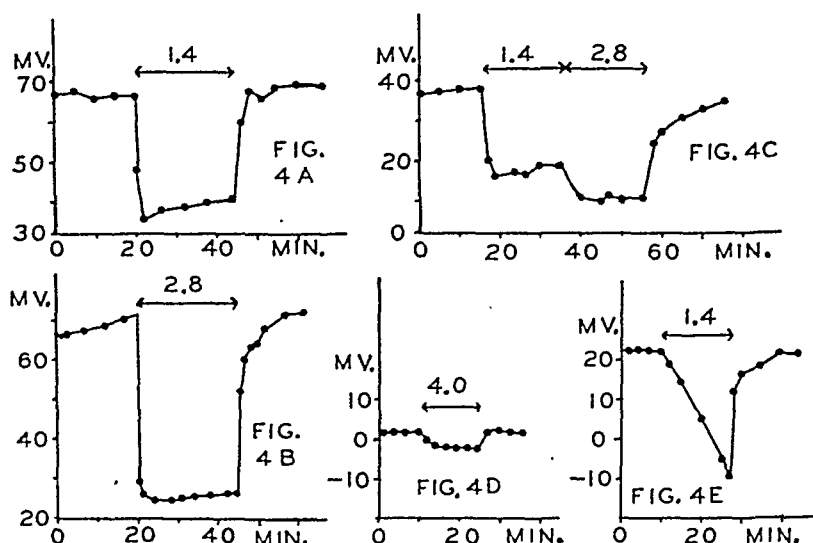


Fig. 4. EXPERIMENTS IN WHICH ELECTROMOTIVE FORCE WAS DETERMINED by momentarily interrupting current and measuring P.D. across stomach with a potentiometer (see text). Period of current flow indicated at top of each figure. Current density in ma. per cm.<sup>2</sup> Fig 4A and B represent experiments performed on non-secreting stomachs; fig. 4C, an experiment performed on a histamine-stimulated secreting stomach; fig. 4D, an experiment performed on a dead stomach and fig. 4E, an experiment on a dead stomach in contact with 0.16 N HCl.

phase was present with current densities of 1.4 ma. per cm.<sup>2</sup> and higher. It was also found that the rapidly rising phase was absent in dead stomachs, as is illustrated in this figure.

Following the rapidly rising phase the P.D. increases relatively slowly, and it was found that at the end of two seconds following the break of the circuit the magnitude of the P.D. in the great majority of cases was not over 5 mv. higher than at the end of the rapidly rising phase. It was found that readings could be obtained with the type K potentiometer in about two seconds and that these values were essentially equal to the magnitude of the P.D. obtained with the string galvanometer at the end of two seconds.

A series of experiments were performed in which the P.D. following momentary breaking of the circuit was measured in this way with the potentiometer. Typical experiments in which this method was used are shown in figure 4. Figures 4A and 4B show experiments performed on non-secreting stomachs and figure 4C an experi-

ment performed on a (histamine-stimulated) secreting stomach. Figure 4D shows the effect of current flow on the P.D. of a dead stomach. A typical experiment is shown in figure 4E in which the ability of a potential originating at the junction of a 0.16 N HCl solution and a dead stomach to produce electrical energy is determined by this method. These experiments will be discussed more fully later on.

*Calculation of the electrical energy output of the stomach.* On the basis of the considerations discussed above, the electrical energy production of the stomach during the passage of a current through the stomach will be at least equal to the P.D., due to the inherent electromotive force following the break of the circuit times the current. Figure 2B represents a typical experiment in which the electrical energy output is plotted against the current density, using both of the above described methods for determining the P.D. (due to  $E_s$  of fig. 1B, or  $E_1$  of fig. 1D). It can be seen in this experiment, using the method in which the rapidly rising phase of the P.D. is disregarded (referred to as the first method in the following), that the electrical energy output reaches a maximum at a current density of approximately 2.0 ma. per cm.<sup>2</sup>. Using this method, the average maximum electrical energy output in experiments performed on 6 dogs was 38 microwatts per cm.<sup>2</sup> with a range of values of from 29 to 44 microwatts.

Similar calculations were made in which the rapidly rising phase of the string record was not disregarded and the P.D. for electrical energy calculations was taken as the initial value of the P.D. on the string record (referred to as the second method in the following). As can be seen in the experiment in figure 2B, the maximum electrical energy production, using this method, was approximately three quarters of that determined by the first method. The actual average was 28 microwatts per cm.<sup>2</sup>, with a range of values from 24 to 30. The magnitude of the current density at the point of maximum electrical energy output was in the neighborhood of 1.0 ma. per cm.<sup>2</sup>. Because of the obvious sources of error in using the initial value of the P.D. of the galvanometer record in making these calculations, it should be pointed out that the above current density is near the level of current density at which the rapidly rising phase of the P.D. first makes its appearance.

In the experiments in which the P.D. was measured after the break of the circuit with the potentiometer, it was found that, using this P.D. in the calculations, the average electrical energy output was found to be 47 microwatts per cm.<sup>2</sup> (with a range of values from 40 to 78 microwatts per cm.<sup>2</sup>).

*Analysis of the possible factors determining the magnitude of the P.D. following a period of current flow.* Although not much is known about the actual factors responsible for electrical transients in living tissues (3, 5), it is possible for convenience of analysis to consider the rapidly rising phase of the P.D. following the break in the circuit to be a function of one or more of the following: *a*) the electrical capacity of the stomach (assuming a static capacity), *b*) changes in the electromotive forces giving rise to the resting P.D. across the stomach wall, *c*) the decay of a back electromotive force produced by the flow of current, and *d*) changes in the resistance of the stomach, i.e., changes in  $R_1$  or  $R_2$  if the circuit is similar to that of figure 1D.

It was assumed in the foregoing that the rapidly rising phase of the P.D. is not due to the discharge of the capacity of the stomach. The objection might be

raised, however, that these transients are of approximately the same duration as those found by Blinks (10) for Nitella and, therefore, might be considered as due to the discharge of a static capacity since Blinks' calculations gave a value of about one microfarad per cm.<sup>2</sup> (11). However, calculations on this assumption give a relatively enormous value for the capacity of the stomach (the resistance of Nitella is of a higher order of magnitude than that of the stomach). A typical calculation illustrating this point is given in the following. The well known formula for the discharge of a condenser was used in the calculations (4, 12).

$$i = \frac{E}{R} e^{-(t/CR)} \quad 3$$

During the flow of current  $I_x$  (fig. 1F) the condenser  $C$  of this equivalent circuit would be charged and the magnitude of the charge on the condenser would be equal to  $R_1 I_x$ . Following the break of the circuit the condenser  $C$  would discharge through  $R_1$ , resulting in a P.D. between A and B of figure 1F in which A would be positive to B. This P.D. between A and B would, therefore, be oriented in the opposite direction to  $E_s$ , and the magnitude of this P.D. at a given instant would be equal to the value of  $E_s$  (extrapolated from the plateau portion of the curve, fig. 2C) minus the actual P.D. of the string record. With these considerations in mind equation 4 can now be written:

$$V_{AB} = IR_1 = R_1 I_x e^{-(t/CR_1)} \quad 4$$

where  $V_{AB}$  is the P.D. between points A and B in volts and at any instant of time is equal to  $IR_1$ , where  $I$  is the current in amperes flowing through  $R_1$ . The initial charge  $E$  on the condenser  $C$  in volts is equal to  $R_1 I_x$ ,  $t$  is the time interval in seconds following the break of the circuit, and  $C$  the capacity in farads. In a typical experiment in which  $I_x$  was 2.0 ma. per cm.<sup>2</sup> the values for  $V_{AB}$  in mv. at given time intervals were found to be: 14.5 mv. at 10 msec., 10.0 mv. at 20 msec., 6.5 mv. at 30 msec., and 2.9 mv. at 50 msec. It was found by substituting these values in equation 4 and solving these equations, that  $C$  is equal to 2260 microfarads per cm.<sup>2</sup> and  $R_1$ , 10.9 ohms per cm.<sup>2</sup>. The total resistance between electrodes  $S_2$  and  $M_2$  was 382 ohms per cm.<sup>2</sup>. This resistance was determined before the above experiment was performed by measuring the P.D. before and during the passage of a very small current (0.03 ma. per cm.<sup>2</sup>) through the stomach and calculating the resistance from equation 1 (assuming  $E_s$  and  $R_1$  are unchanged by this current). The resistance from  $S_2$  to  $M_2$ , measured without the stomach, was 137 ohms per cm.<sup>2</sup>. Hence the resistance of the stomach ( $R_1 + R_2$  of fig. 1F) would be 245 ohms per cm.<sup>2</sup>.

Substitution of the values for  $C$  and  $R_1$  obtained above in equation 4, yields an equation that gives an exceptionally good fit to the P.D.-time curve up to the plateau portion of the curve. It should be pointed out that, if an arbitrary value of  $R_1$  is used such as the total resistance of the stomach (5, p. 26) and the first pair of values ( $t = 10$  msec. and  $V_{AB} = 14.5$  mv.) substituted in the equation, the value of  $C$  is lower than that obtained above but the resulting equation fits the actual curve very poorly (curve B of fig. 2C). The values for  $C$ , calculated from the other experiments, reveals that the values are all of the same order of magnitude as that given in the ex-

ample above. It might be concluded, therefore, because of the relatively large calculated value of  $C$  that the rapidly rising phase of the P.D. is not due to the discharge of a static capacity, but to one or more of the other factors enumerated above. However, if the effective surface of the stomach is considered as including the total area of the crypts and tubules, then the capacity of a  $\text{cm}^2$  of this surface would be much less than that calculated above and  $R_1$  would be much larger, so that it is possible that the rapidly rising phase may be considered as due to the discharge of the capacity. It should be pointed out that the rapidly rising phase of the P.D. is not present in the dead stomach (fig. 3), but is still present in the intact stomach after the flow of large currents has reduced the P.D. to very low values (fig. 3).

Assuming that the P.D. following the break of the circuit is due to the sum of a polarization potential  $E_p$  of figure 1F and  $E_s$ , the electrical energy output of the stomach would be equal to  $(E_s - E_p)I_x$ . Actually the main interest of the authors is in the ability of  $E_s$  to produce electrical energy and, therefore, the P.D. used to calculate the electrical energy output of  $E_s$  would be equal to the P.D. of the galvanometer record plus the value of  $E_p$ . It should be mentioned that  $E_p$  could conceivably contribute to the P.D. across the stomach for a longer period than the duration of the rapidly rising phase of the P.D. For example, it is possible that the value of the P.D. measured five seconds following the break of the circuit is a more accurate measure of  $E_s$  than either of the above methods. If this value is used in calculating the electrical energy output, then the output of  $E_s$  was found in some experiments to be over 120 microwatts per  $\text{cm}^2$ .

It is not possible on the basis of the present data to determine rigorously the contributions of the various factors given above to the actual P.D.-time curves. Although there would be many difficulties, it is possible that further experimentation might throw more light on the actual factors responsible for the P.D.-time curves. One obvious and not too difficult approach would be to determine whether or not the flow of current results in the production of a P.D. across the outer muscle layers of the stomach. If it did, this P.D. could not be due to changes in the electromotive force giving rise to the resting P.D. of the stomach, since it has been shown (13) that this electromotive force originates somewhere between the submucosa and mucosal surfaces.

#### DISCUSSION

*Comparison of the electrical energy output with the minimum free energy necessary for the production of HCl.* Comparison of these two characteristics reveals that the electrical energy output of the resting stomach determined by the above methods is definitely less than the energy needed for the production of HCl. For example, in a typical experiment the maximum electrical energy output of the resting stomach (determined by the first of the above methods) was found to be 39 microwatts per  $\text{cm}^2$ . Following histamine stimulation this same stomach secreted HCl at a maximum rate of 0.013 mg. per  $\text{cm}^2$  per minute. Previous calculations (9) have shown that the minimum free energy needed for the production of 1 mg. of HCl is equal to approximately 1.1 joules, so that the minimum free energy needed to produce 0.013 mg. of HCl would be approximately 0.014 joule. Converting this latter value to

microwatts, it is found that the minimum rate of production of free energy needed to produce HCl is 233 microwatts per  $\text{cm}^2$ . Therefore, in this experiment the electrical energy production determined by the first of the above methods is found to be only about one sixth of the minimum amount needed for HCl production. Using the second method for determining the electrical energy production, the amount of electrical energy produced would be only about one ninth of the minimum amount needed for HCl production. Two similar experiments were performed with essentially the same results. The rate of HCl secretion found in these experiments was less for unknown reasons than the average rate of secretion found in previously published experiments (7, 9, 14). If the average rate of secretion of HCl from previous experiments is compared to the average rate of production of electrical energy of the present experiments, it is found that the electrical energy production, instead of being one sixth or one ninth of the minimum free energy needed for the production of HCl, is closer to one fourteenth or one twentieth.

In the above experiments the electrical energy production was determined on non-secreting stomachs. It is possible that the electrical energy output of the secreting stomach might be greater than that of the resting stomach. A few experiments were performed on secreting stomachs (3 dogs) in which the P.D. following the break of the circuit was measured with the potentiometer. It was found with this method (fig. 4C) that the electrical energy output of the secreting stomach was not markedly different from that found by the same method for the resting stomach.

Before the conclusion is warranted that there is not enough electrical energy available for the production of HCl, it would have to be shown that the methods used in the present experiments are adequate methods for the determination of the total electrical energy production of the stomach. As pointed out above, the methods used in the present work will at best only determine the ability of those electromotive forces to produce electrical energy that are oriented in such a way as to contribute to the P.D. across the stomach. If there are locally completed circuits within the stomach, as illustrated in figure 1E, then the amount of electrical energy produced would be greater than that determined by the present methods. Furthermore, if there are electromotive forces oriented so that they do not contribute to the P.D. across the stomach, the present method would not determine the ability of these electromotive forces to produce electrical energy.

It is conceivable that there are electromotive forces oriented across the cells of the gastric tubules toward the lumen of these tubules which would not contribute (or would contribute only a small component of their electromotive force) to the P.D. across the stomach, and that the ability of these electromotive forces to produce electrical energy may be many times (because of the relatively large surface area ratio) the ability of those electromotive forces to produce electrical energy that are oriented so as to contribute to the P.D. across the stomach. Therefore, an answer to the question as to whether or not the stomach electromotive forces can produce enough electrical energy for the production of HCl will have to await further experimentation.

*Analysis of the electrical energy theory of osmotic work.* An implication of the theory that the stomach uses electrical energy for the production of HCl is that there

must be completed circuits inside the stomach, otherwise the stomach could only produce a very small fraction of the amount of electrical energy needed. The authors are unaware of any evidence in the literature demonstrating that, in tissues such as the stomach with a maintained P.D. across them, there are locally completed circuits (apart from the current that would flow between adjacent macroscopic areas of different P.D. through the fluid or tissues external to the mucosa of the stomach). In fact, attempts to test this important part of the hypothesis will probably be attended with great difficulties. The electrical energy theory of osmotic work would, therefore, have to postulate a circuit similar to the one shown in figure 1E. In previous work (9, 15) it has been shown that the flow of current from serosa to mucosa results in an increase in the production of HCl in the secreting stomach, and that the flow of current in the opposite direction results in a decrease in the production of HCl. With these facts in mind, it would be reasonable to postulate that the flow of current from *K* to *L* in figure 1D would result in the production of HCl in some part of the circuit between *K* and *L*. Since the flow of current through a resistance could not provide energy that can be used for useful work (9) it would have to be further postulated that there is a back electromotive force at the locus where electrical energy is transformed into osmotic work. The circuit in figure 1G is a circuit that would fulfill the above requirements. In this circuit  $E_H$  represents the locus where HCl is formed, and it is assumed that  $E_s$  is greater than  $E_H$ . A somewhat similar theory has been formulated by Crane, Davies, and Widdowson (16).

Apart from the problem of the production of HCl, there are some interesting implications of the present findings that will be discussed below.

*Comparison of the electrical energy production of the stomach with that of the electric organ of electrophorus electricus.* Nachmonsohn *et al.* (17) found, under their experimental conditions, that the electrical energy production of the electric organ is  $67 \times 10^{-6}$  joules per gm. of tissue per impulse. They found that the electric organ was fatigued when it discharged at a rate of 800 impulses per minute. The electrical energy output at this rate of discharge would be equal to 0.052 joules/gm/min. In a later paper (18) a higher rate of electrical energy production was found, i.e. 0.085 joules per gm. per minute (data from tables 1, 2, and 5 of their paper). The average electrical energy output of the mucosa of the stomach in comparable units was calculated and found to be 0.012 and 0.0088 joules per gm. of mucosa per minute by the two methods for determining electrical energy output. It can be seen that the electrical energy output of the stomach is approximately from one fourth to one tenth that of the electric organ. It should be kept in mind, however, that the values for the electric organ were obtained under conditions in which the electric organ was becoming fatigued, while the values for the electrical energy output of the stomach were obtained during a relatively steady state. It is possible that the maximum electrical energy output of the electric organ during a steady state might be much closer to the output of the stomach than the above figures would indicate.

Because of the high voltage produced by the electric organ it might seem unlikely at first glance that the electrical energy output of the electric organ is not markedly different from that of the stomach. However, it must be recalled that the total mass of the electric organ is relatively large and that the voltage of a cross section of the



organ weighing one gram would be a small fraction of the total voltage. Furthermore, a discharge of the electric organ lasts for only a few msec. which, at the rate of discharge of 800 per minute, means that the current would be flowing for only a small fraction of the time, while in the case of the stomach the current is flowing continuously.

*Maximum current density produced by the stomach.* The present experiments indicate that the electromotive force giving rise to the P.D. across the stomach can produce a current density of approximately one ma. per cm.<sup>2</sup> (or 3 ma. or more if the first method is used). This finding is of interest in the light of the recent work of Marsh (19) in which he demonstrated that the growth of neuroblasts *in vitro* can be controlled by the flow of electric currents. The threshold from this effect was in the neighborhood of 10 ma. per cm.<sup>2</sup>. An implication of these findings is that the flow of electric current inside the developing organism might control the direction of growth of neuroblasts. The obvious objection to this hypothesis, that the electromotive force of tissues could not produce a continuously maintained current of this magnitude, is somewhat negated by the findings in the present work. On the assumption that there are completed circuits inside the stomach, the density of the current flowing in a given direction, assuming uniform resistances, would be twice the current densities given above. Furthermore, if the resistances of the stomach were not uniform then the current density in certain regions might be much higher, i.e. as high as 10 ma. per cm.<sup>2</sup> While the characteristics of the stomach may be quite different from those of embryonic tissues, the present findings indicate that the electromotive force of living tissues can produce maintained currents of relatively high magnitudes.

These findings also raise the interesting question as to the mechanism responsible for the production of the electromotive force. Most investigators have attempted to account for the maintained P.D. across tissues on the basis that the potential arises from unequal ion mobilities (20) or unequal ion solubilities (21). Lund (22), on the other hand, has suggested that these maintained P.D.'s are oxidation reduction potentials. An implication of Lund's theory is that cell membranes may act as first-class conductors (23, 24). The findings in the present work raise the question as to whether these various types of potentials can give rise to currents of the magnitude that the electromotive force of the stomach can produce. Obviously from our knowledge of oxidation-reduction cells (lead storage cells, etc.), it is evident that oxidation-reduction batteries can give rise to currents of much higher magnitudes than those produced by the stomach. The writers have not been able to find data in the literature on the maximum ability of electromotive forces, depending on unequal ion mobilities or solubilities, to produce electric currents. A preliminary attempt to obtain an idea of how much electrical current these latter potentials can produce was made by studying the effect of current flow on the potential produced by applying 0.16 N HCl to a dead stomach. A typical experiment is shown in figure 4E. The P.D. across the dead stomach, when 0.16 N HCl was applied to the mucosa, was found to be approximately 20 mv. It can be seen from figure 4E that with a current density of 1.4 ma. per cm.<sup>2</sup> the P.D. rapidly declines to zero and becomes inverted. In other words, the electromotive force of this system (due undoubtedly to unequal ion mobilities or solubilities, or both) cannot give rise to the current densities pro-

duced by the living stomach. It might be argued that the decrease in the P.D. in this experiment was due to mixing of buffers from the dead stomach with the HCl solution, with a consequent reduction of its hydrogen ion concentration. That this was not the case is shown by the behavior of the P.D. following the period of current flow. It can be seen from figure 4E that the P.D. returned to approximately its original magnitude following this period. Also it was found that the pH of the HCl solution was not significantly changed following such an experiment.

There are many objections, however, against concluding that the stomach electromotive force must therefore be due to oxidation-reduction potentials. For example, it is possible that the effective surface of the living stomach may be much greater than that of the dead stomach, and therefore the density of the current flowing across the surface of the living stomach for a given current density in the external circuit may be much less than for a dead stomach. Also further work needs to be done on the ability of potentials due to different ion mobilities or solubilities to deliver current in which other species of ions and other interfaces are used. Nevertheless the present experiments offer a real challenge to those investigators who are interested in the mechanism of the production of the P.D.'s of living tissues.

#### SUMMARY

An attempt was made to measure the ability of the stomach to produce electrical energy by a method in which an external battery is connected in series with the stomach. It is shown that the electrical energy produced by the stomach under these conditions is at least equal to the product of the P.D. across the stomach due to the electromotive force following the break of the circuit and the magnitude of the current flowing through the stomach.

The P.D. due to the electromotive force was determined by momentarily breaking the circuit and measuring with a string galvanometer the P.D. across the stomach wall. With higher current densities a rapidly rising phase of the potential difference was found to be present in the galvanometer records. Calculations of the electrical energy production were made on the assumption that the rapidly rising phase of the P.D. was due to *a*) the decay of a back electromotive force produced by the flow of current and *b*) a rapid change in the inherent electromotive force of the stomach. In the first method the level of the P.D., following the rapidly rising phase, was used to calculate the electrical energy production by the inherent electromotive force of the stomach, and in the second method the initial reading of the P.D. was used. It was found by the use of these two methods that the average electrical energy production by the stomach was 38 and 28 microwatts per cm.<sup>2</sup>, respectively. The possible factors responsible for the rapidly rising phase are discussed.

The electrical energy output of the stomach, with the above methods, was found to be definitely less than the minimum free energy necessary for the production of HCl. It was also shown, however, that the methods employed in the present work would underestimate the ability of the stomach to produce electrical energy if one or both of the following conditions are present: *a*) electromotive forces oriented in such a way as not to contribute to the P.D. across the stomach wall and *b*) locally completed circuits inside the stomach. It is concluded that it is not possible at pres-

ent to decide as to whether the stomach can produce enough electrical energy for the production of HCl.

The findings of the present work indicate that the electromotive force of the stomach can produce a continuous current of around 1 ma. per cm.<sup>2</sup> (or current densities of several ma. per cm.<sup>2</sup> on the basis of assumption *a* above). The implications of these findings are discussed. It is pointed out that these findings may throw light on the mechanism of the production of the electromotive force. It is shown that a potential produced by the application of 0.16 N HCl to a dead stomach (a potential undoubtedly depending on different ion mobilities or solubilities) cannot produce continuously maintained currents of the magnitude produced by the living stomach.

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# SALT EXCRETION IN DESERT MAMMALS<sup>1</sup>

KNUT SCHMIDT-NIELSEN, BODIL SCHMIDT-NIELSEN AND  
HOWARD SCHNEIDERMAN

*From the Martin Biological Laboratory, Swarthmore College*

SWARTHMORE, PENNSYLVANIA

THE excretion of water from the animal body is regulated so that the water content of the body is kept relatively constant. If the intake of water is restricted, the response is less excretion of water through the kidneys, or, in other words, the urine will be more concentrated with respect to the solid excretory products.

Krogh (1) says: "The organs of excretion are in the higher vertebrates developed to serve the function of conservation of water." Krogh discusses the salt concentrations found in vertebrate urine from the viewpoint of water conservation in animals with restricted water supply. He finds that the highest urine concentrations are observed in whales, which have a heavy physiological load because their only source of water is the food organisms. Recently it has been demonstrated (2, 3) that certain desert animals have a considerably more efficient excretory system.

In view of those results we wanted to test the excretory ability in these desert animals with respect to electrolytes and chlorides, by imposing a heavy load of sodium chloride through the diet.

The animals used were two species of the family Heteromyidae, the kangaroo rat (*Dipodomys merriami*) and the pocket mouse (*Perognathus baileyi*). These animals can live indefinitely without drinking water and gain weight on a diet of dry grain only. The extra load with sodium chloride was accomplished by feeding grain containing 10 per cent by weight of NaCl.

1000 gram rolled barley was soaked in 2.8 liters of a 10 per cent NaCl solution. After 24 hours 1.8 liters could be drained off, which means that 1000 ml. solution (100 gram NaCl) was left in the grain. Then the grain was dried at 105° C., leaving about 10 per cent NaCl by weight in the dry food.

## EXPERIMENTAL RESULTS

We used five *Perognathus* for testing the maximum salt excretion in the urine. The animals had lived for five to six weeks on a diet of dry grain only and no water before the experiment was started. The four of them had gained considerable weight in this preliminary period. They were then transferred to the salt grain diet for two days, and subsequently to dry grain again. During the salt grain diet all animals lost weight (aver. 8 per cent), but it is amazing that after the severe strain of

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Received for publication June 29, 1948.

<sup>1</sup> This work was supported by funds from Air Material Command, Wright Field, and facilitated by the interest in the problems shown by the Aero Medical Laboratory. It was extended and completed by the support received from the Office of Naval Research.

the excessive salt intake the animals were able to gain weight again on a completely dry diet. In two days the average gain was nearly 3 per cent and they continued to increase in weight. Most of these animals were still alive six months later, and they were fat and healthy.

We do not know how much of the food was consumed by the animals. (Exact studies of food consumption are made fairly complicated in these animals because they store food in their cheekpockets.) However, a considerable amount of the grain was actually eaten, and as a consequence the urinary salt concentrations increased tremendously.

The figures in table 1 give the chloride concentrations in the urine the second day on the salt diet. All the animals had very high chloride concentrations and electrolyte concentrations in the urine. When the animals are on their natural diet chlorides ordinarily make up only a small fraction of the electrolytes in the urine. However, on the salt diet about three fourths of the electrolytes were chlorides.

TABLE 1. URINE CONCENTRATIONS IN POCKET MICE AFTER TWO DAYS OF EXCESSIVE SALT INTAKE

DAYS ON DRY GRAIN	WT. LOSS, % AFTER 2 DAYS ON SALT GRAIN	WT. GAIN, % AFTER 2 DAYS ON DRY GRAIN	URINE CONCENTRATIONS AFTER 2 DAYS ON SALT GRAIN DIET		
			Chloride	Electrolytes	Urea
			mN	mN	mN
35	7.7	3.9	762	1070	2610
35	8.2	2.4	—	—	—
34	8.3	1.9	845	1140	1730
43	8.9	3.7	758	980	1270
44	9.1	2.2	515	1160	1570
Av.: 38	8.4	2.8	720	1088	1795

The urea concentrations are considerable but do not reach excessively high values. All urea concentrations are higher than can be reached by man, and the highest value is a little higher than can be reached by the white rat. It is clear that the urea excretion to a considerable degree can be maintained simultaneously with high electrolyte excretion. A simultaneous load with salt and urea was never tried, but would surely give interesting results.

As mentioned above the animals recovered again on a diet of dry grain only, so the excessive salt load had no ill effects that could not be repaired. There is no doubt, however, that the animals are unable to survive on the salt grain diet.

The pocket mice lost considerable weight in two days and would not have been able to survive for a long period. In a group of kangaroo rats which was given the salt grain the longest surviving individual lived for 17 days (table 2).

Most of the kangaroo rats died after about a week, but even this length of survival must be considered a remarkable accomplishment. It might be expected that the animals, after an initial period, practically stopped eating and died from partial starvation. However, when completely starved the kangaroo rats live for only three days or less (seven individuals kept without food died after 1, 2, 2, 3, 3,

3, and 3 days, respectively). We noticed, much to our surprise, that the animals did not show any high degree of desiccation at the time of death (table 2). Ordinarily

TABLE 2. SURVIVAL TIME OF KANGAROO RATS ON A DIET CONTAINING 10% NaCl

DAYS SURVIVAL ON SALT GRAIN DIET	WT. LOSS	WATER CONTENT AT DEATH; PERCENTAGE OF BODY WEIGHT
	%	
2	14.8	69.2
4	14.7	69.5
4	21.3	68.3
5	22.1	68.9
6	25.9	67.8
6	26.9	68.6
7	24.8	70.6
7	28.4	67.2
8	23.2	67.5
11	33.6	69.4
17	36.6	68.8
Av.: 7	24.8	68.7

TABLE 3. URINE AND PLASMA CONCENTRATIONS IN KANGAROO RATS FED GRAIN CONTAINING 10% NaCl

DAYS ON SALT GRAIN DIET	WEIGHT LOSS	WATER CONTENT	URINE			PLASMA		
			Chloride	Electrolytes	Urea	Chloride	Electrolytes	Urea
	%	%	mN	mN	mN	mN	mN	mN
2	10.9	68.4	507	625	810	142	158	11.3
	11.5	69.9	773	805				13.4
	15.5	69.7		808	3,120			11.8
	16.7	67.4	728					
	17.6	70.6	908	1,220	2,090		157	26.3
Av.:	14.4	69.2	729	865			159	15.7
6	11.3	68.0	651	1,000		121	154	
	15.2	69.2		1,010		122	158	
	16.4	68.5		537		111	153	
	19.1	69.0	586	776		123	162	
	20.8	67.7				155	163	
	26.6	68.8	229	1,000		135	162	21.5
Av.	18.2	68.5		865		128	159	

an ingestion of large amounts of salts causes heavy diarrhea and dehydration. Diarrhea was not observed in these animals.

Excretion from the kidneys was much alike in the kangaroo rats and the pocket mice. We found some extremely high urinary concentrations (table 3; the table is incomplete because of difficulties in obtaining urine samples large enough for all

analyses.) The data include the highest chloride value ever found in urine, 908 mN (equal to 5.3 per cent NaCl). In the same sample we have the highest concentration of electrolytes, 1220 mN, which is more than twice as concentrated as sea water. Adolph (4) gives the following maximum urinary concentrations of chloride: rat, 600 mN; man: 370 mN; dog: 330 mN; goat: 320 mN.

Irving *et al.* (5) were interested in the water balance of the seal. Since the seal has no access to drinking water it has quite an interesting water problem. It turned out that the seal can maintain the water balance with the water of the food (fish) because of the fairly high water content in the fish and the low evaporation from the seal itself. As for the possibility of using sea water for drinking Irving and colleagues say "The kidney which could abstract water from a 3.5 per cent salt solution would be performing osmotic work at an amazing intensity." This is truly so, and we can add that we were very much astonished to find an excretory system with twice that performance.

We have some samples from kangaroo rats which had lived for more than two days on the salt diet. Also here we find some high urinary concentrations, but we do not have many simultaneous figures for urine and plasma. We give a table of some figures obtained. The plasma seems not to be excessively concentrated with respect to electrolytes, while the chloride values undoubtedly are above the usual level.

#### SUMMARY

Some desert rodents excrete a very concentrated urine, which enables them to expend only small amounts of water for excretion. The maximum excretory ability with respect to electrolytes is about 1200 mN, and for chlorides about 900 mN. This appears to be far in excess of the limits known from other mammals. This ability must be interpreted as a very useful mechanism for water conservation and an adaptation to desert life.

This work is part of a project for physiological field research initiated by suggestions from Dr. L. Irving. The experimental work was carried out at the Santa Rita Experimental Range, Arizona, and we greatly appreciate the permission to work at the station given by Mr. R. Price, Director, Southwestern Forest and Range Experiment Station, Tucson, Ariz.

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# EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CLEARANCE OF THE DALMATIAN DOG<sup>1</sup>

MEYER FRIEDMAN AND SANFORD O. BYERS

*From the Harold Brunn Institute, Mt. Zion Hospital*

SAN FRANCISCO, CALIFORNIA

IN A previous study (1), it was found that the administration of salicylate to rats produced an increase in their renal excretion of uric acid. Blood analyses and renal clearances done on these rats receiving salicylate, however, indicated that the observed increase in uric acid excretion was not preceded or accompanied by an increase in 1) blood uric acid, 2) glomerular filtration rate or 3) in renal plasma flow. These observations suggested that salicylate increased the excretion of uric acid by impeding the latter's renal tubular reabsorption. Talbott (2) previously had come to the same conclusion concerning the mechanism of action of salicylate in the human subject.

In view of the fact that recently we (3) discovered that the Dalmatian dog excreted uric acid at the level of glomerular filtration without subsequent tubular excretion or reabsorption, it seemed important to determine the effect of salicylate upon the excretion of uric acid in this species of dog. For if salicylate actually increases the output of uric acid in mammals by impedance of its tubular reabsorption, this drug should not be uricosuric in the Dalmatian dog, as the renal tubules of this animal normally do not reabsorb the uric acid present in the glomerular filtrate. The results of such a study are reported herein.

## METHODS

Two healthy, male, thoroughbred Dalmatian dogs (litter mates) weighing approximately 20 kilos. each and 6 male mongrel dogs of approximately the same size were used in this study. Five uric acid and creatinine clearances were done on the 2 Dalmatians both before and during the intravenous injection of sodium salicylate. Eight similar combined clearances were done on the 6 mongrel dogs both before and during salicylate injection.

All dogs were anesthetized with pentobarbital sodium, catheterized and given a solution of 0.9 per cent  $\text{Na}_2\text{SO}_4$  by vein at the rate of 4 cc. per minute until a urine flow of at least 1 cc. per minute occurred. The dogs then received an intravenous infusion of a solution containing 4 mg. of creatinine per cc. The animals received 100 cc. of this latter solution within 5 minutes, after which time they continued to receive it at the rate of 4 cc. per minute. After 30 minutes, the bladder was emptied, a blood sample taken and the first urine collection was begun. After 15 minutes, the bladder was emptied, a second blood sample taken and a second urine collection of 15 minutes was obtained in the same manner. After the second control urine collection had been taken, a solution containing 10 mg. of sodium salicylate, as well as 4 mg. of creatine per cc., was given by intravenous infusion at the rate of 4 cc. per minute for the remainder of the experiment. Each dog received approximately a total of 3.5 gm. of sodium salicylate. Thirty minutes after this last infusion had begun, a third and fourth urine collection (each of 15 minutes duration) with appropriate blood

Received for publication July 6, 1948.

<sup>1</sup> Aided by Grants from The Public Health Service and The Wine Advisory Board of the State of California.



samples were taken exactly as described in the control period. Determinations of the uric acid and creatinine in the blood and urine samples allowed the calculations of the uric acid and creatinine clearances.

Uric acid in plasma and urine was determined according to the method of Folin (4). Creatinine determinations were done according to the method of Folin and Wu (5).

TABLE 1. EFFECT OF SODIUM SALICYLATE UPON THE URIC ACID CLEARANCE OF THE DALMATIAN AND NON-DALMATIAN DOG

DOG	BEFORE SALICYLATE					AFTER SALICYLATE				
	UV <sup>1</sup>	PUA <sup>2</sup>	UAC <sup>3</sup>	CC <sup>4</sup>	UAC/CC	UV <sup>1</sup>	PUA <sup>2</sup>	UAC <sup>3</sup>	CC <sup>4</sup>	UAC/CC
<i>Dalmatian dogs</i>										
<i>D1</i> . . . . .	4.7	0.38	99.0	99.0	1.0	4.5	0.40	87.2	88.0	0.99
<i>D1</i> . . . . .	5.4	0.40	82.0	86.0	0.96	3.6	0.38	84.0	89.0	0.95
<i>D1</i> . . . . .	3.7	0.47	73.0	66.0	1.10	2.5	0.54	71.0	62.0	0.82
<i>D2</i> . . . . .	6.5	0.53	114.0	98.7	1.15	5.6	0.44	112.5	88.7	0.93
<i>D2</i> . . . . .	7.0	0.43	108.5	112.5	1.05	4.0	0.65	95.8	91.0	1.05
Av. . . . .	5.4	0.44	95.2	92.4	1.05	4.0	0.48	90.1	83.7	0.95
<i>Non-dalmatian dogs</i>										
<i>C1</i> . . . . .	8.1	0.22	28.9	86.0	0.32	7.2	0.17	48.4	83.0	0.58
<i>C1</i> . . . . .	5.9	0.26	16.6	93.1	0.18	7.4	0.24	23.6	88.2	0.27
<i>R2</i> . . . . .	7.5	0.22	31.7	104.0	0.30	4.3	0.23	33.5	105.0	0.32
<i>R2</i> . . . . .	6.0	0.14	43.0	101.0	0.43	3.5	0.16	39.0	100.0	0.39
<i>R3</i> . . . . .	7.1	0.23	21.9	95.0	0.23	4.4	0.23	42.5	83.0	0.51
<i>N1</i> . . . . .	2.2	0.29	34.7	92.0	0.38	4.5	0.34	54.1	97.0	0.56
<i>R4</i> . . . . .	3.6	0.18	30.0	98.0	0.31	3.4	0.21	37.5	107.0	0.35
<i>S1</i> . . . . .	5.5	0.16	23.0	106.0	0.22	3.3	0.22	38.0	106.0	0.38
Av. . . . .	5.7	0.21	28.7	96.9	0.30	4.75	0.23	39.6	96.2	0.42

<sup>1</sup> Equals cc. of urine/min.    <sup>2</sup> Equals mg. of uric acid/100 cc. of plasma.    <sup>3</sup> Equals uric acid clearance in cc/min.    <sup>4</sup> Equals creatinine clearance in cc/min/sq. M. of S.A.

## RESULTS

### A. Effect of Sodium Salicylate Upon 1) The Plasma Uric Acid, 2) Uric Acid Clearance and 3) Creatinine Clearance

1) *Non-Dalmatian dogs*. As table 1 demonstrates, no significant change occurred in the plasma uric acid content of non-Dalmatian dogs after the infusion of salicylate. The average plasma content was 0.21 mg. per 100 cc. before, and 0.23 mg. during, the injection of salicylate.

The uric acid clearance, however (table 1), increased significantly after infusion of salicylate had been started. Thus the average uric acid clearance of the 6 non-Dalmatian dogs was 28.7 cc. per minute during the control period and 39.6 cc. per minute after the administration of sodium salicylate. Despite the decrease in urine volume (table 1) the creatinine clearance remained unchanged after the infusion of sodium salicylate had been started. Accordingly, the uric acid clearance/creatinine clearance changed from 0.30 (before salicylate) to 0.42 after salicylate had been given.

2) *Dalmatian dogs*. Similar to the findings in the non-Dalmatian dogs, the plasma uric acid content of the Dalmatian was not changed (table 1) by the infusion of sodium salicylate. Likewise, there was no significant change in the uric acid clearance after the administration of salicylate. Thus (table 1) the average uric acid clearance was 95.2 cc. per minute before and 90.1 cc. after the injection of salicylate.

Similarly the average creatinine clearance (92.4 cc. per minute) did not change significantly after injection of salicylate. As was observed previously (3) the average creatinine clearance of these dogs was approximately the same as their average uric acid clearance. The uric acid clearance/creatinine clearance ratio therefore remained approximately at unity (table 1) throughout the experiment. A rather marked decrease in the rate of excretion of urine however was noted after the salicylate infusion had been maintained for over 30 minutes. This latter decrease noted in both the Dalmatian and non-Dalmatian was thought to be due to the hypertonicity of the salicylate infusion.

#### DISCUSSION

In a previous study (1), uric acid was found to be increased in the urine of rats after the administration of sodium salicylate. It was thought that this uricosuric effect of salicylate was due to its probable ability to impede the reabsorption uric acid by the renal tubules. In a later study (3), however, the renal tubule of the Dalmatian dog peculiarly was found not to reabsorb or excrete uric acid, the latter substance being excreted at the level of glomerular filtration (i.e., equal to the excretion rate of creatinine). Therefore, if salicylate exerts its uricosuric effect by tubular interference in the reabsorption of uric acid, its action should not be uricosuric in the Dalmatian dog.

The observations of this present study indicate that whereas sodium salicylate has a clear uricosuric action in the ordinary mongrel dog, it has none in the Dalmatian dog. We believe this fact not only confirms our earlier observation concerning the uniqueness of the Dalmatian kidney with respect to excretion of uric acid, but also furnishes further evidence that the uricosuric effect of salicylate, when manifested, is due to the tubular actions of the drug.

#### CONCLUSIONS

The administration of sodium salicylate was not able to effect a change in the uric acid clearance of the Dalmatian dog. The significance of this observation in relation to the renal peculiarity of the Dalmatian dog and the mode of action of salicylate was discussed.

The authors express their thanks to Ann Miller and Catherine Shuey for technical assistance in the execution of this study.

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## MEASUREMENT OF RENAL FUNCTIONS IN RATS

A. C. CORCORAN, GEORGES MASSON, RUTH REUTING, AND IRVINE H. PAGE

*From the Research Division of the Cleveland Clinic Foundation*

CLEVELAND, OHIO

**R**ECOGNITION of substances which stimulate growth of renal tissue and the activity of renal enzyme systems initiated the development of a method suitable for bio-assay of such materials from measurements of specific renal excretory functions in rats. The functions selected were the maximum tubular excretory capacity for p-aminohippurate ( $Tm_{PAH}$ ) and the plasma creatinine clearance ( $C_{CR}$ ).  $Tm_{PAH}$  is taken as measuring activity of tubular tissue and  $C_{CR}$  as equivalent to the rate of glomerular filtration.

Experience in the study of renal function in other species led to the premises that the method chosen should involve *a*) complete collection of urine during a state of rapid flow, while *b*) the plasma concentrations of PAH and creatinine during urine collection should be accurately estimated and *c*) the animal maintained under physiological conditions during the test.

Methods hitherto described (1-6) did not fulfill these criteria. The procedure selected is therefore described.

### PROCEDURE

*Apparatus.* *a*) Rat holder (fig. 1); *b*) blood collection pipettes (fig. 1). These are drawn from soft glass tubing 4 mm. I.D.; *c*) ureteral catheter, no. 4 F, cut 6 inches in length; and *d*) box for heating at 45° C.

*Solutions.* *a*) Heparin in 0.9 per cent NaCl; 1 cc. contains 2 mg. heparin. *b*) PAH-mannitol-creatinine: sodium p-aminohippurate 6 gm.; mannitol 10 gm.; creatinine, 4 gm.; 0.9 per cent NaCl q.s. to 100 cc. *c*) Intracaine (Squibb) 2.5 per cent in 0.9 per cent NaCl.

*Experimental.* A female rat weighing 170 to 250 gm. was injected intraperitoneally with 0.3 cc. of heparin solution. The catheter was inserted into the bladder under light ether anesthesia. PAH-mannitol-creatinine solution was injected subcutaneously in two equal doses of 1.7 cc. per 100 gm. body weight. The time of this injection was noted as 0. The rat was then placed in the holder, adjusted so as to restrain movement (fig. 2). The position of the catheter was checked by observing urine flow. Discomfort from its presence was minimized by injecting 0.2 cc. of intracaine solution into the bladder, retaining it there for several minutes. Nearly all animals soon accustom themselves to the procedure and remain quiet during the period of observation. Those few which do not are rejected.

At 40 to 43 minutes after 0 time, rat and holder were warmed at 45°C. for 3 minutes in a box heated by an electric light bulb to this temperature. At 45 minutes the first sample of blood ( $B_1$ ) was collected by snipping the end of the tail and milking

out about 0.4 cc. of blood onto a heparinized watchglass. The blood was drawn into the pipette and the drawn-out end sealed in a flame. At 48 minutes the bladder was rinsed with four successive 0.2 to 0.5 cc. volumes of saline, the last rinse ending exactly at 50 minutes. These rinses were discarded. Urine collection for clearance measurement was begun at 50 minutes by directing the catheter into a small graduated cylinder. The bladder was rinsed in the same manner, beginning at 58 and ending exactly at 60 minutes. These rinsings were added to the urine and the mixture of

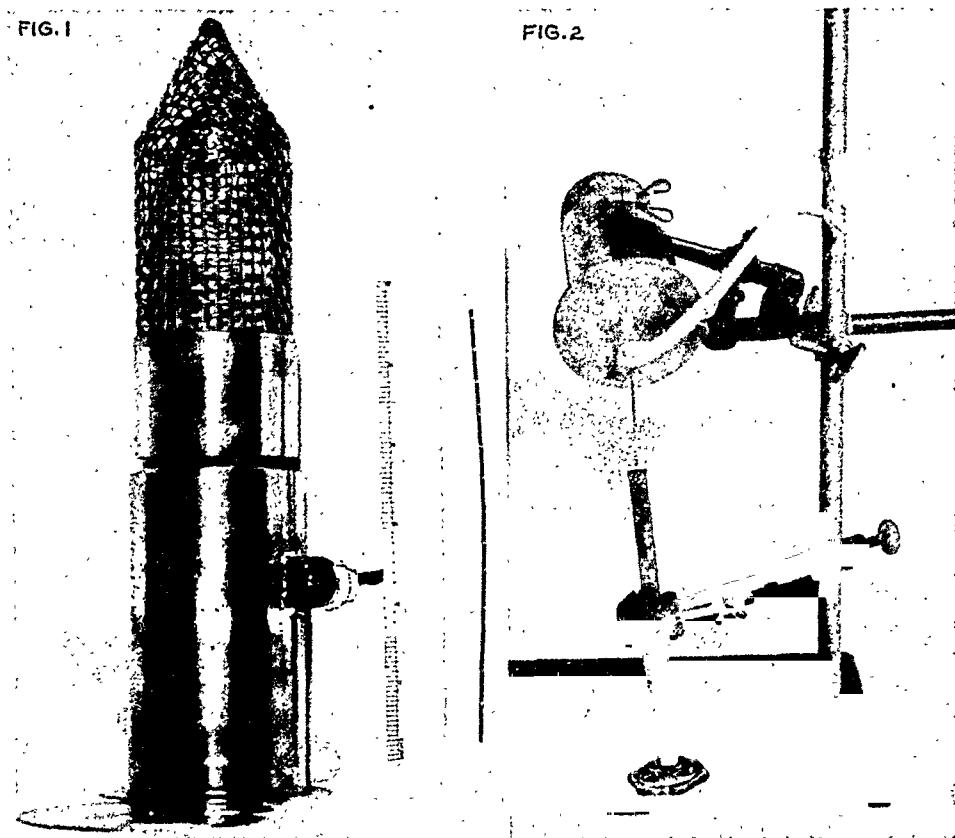


Fig. 1. RAT HOLDER CONSTRUCTION, blood pipette and catheter to scale  
Fig. 2. RAT IN HOLDER during urine collection

urine and bladder rinse made up to 100 cc. in a volumetric flask. The rat was again warmed and blood collected (B<sub>2</sub>) at 65 minutes.

Blood samples were then centrifuged, the pipettes scored with a diamond point at the cell-plasma interface, the pipette broken and 0.1 cc. of clear plasma taken up in a volumetric pipette. A protein-free filtrate (1/100 dilution of plasma) was made by cadmium sulfate-sodium hydroxide precipitation. PAH was determined in filtrate and diluted urine by the method described by Goldring and Chasis (7) and creatinine by an adaptation of the method of the familiar alkaline picrate procedure. Mean plasma concentration during urine collection was found by interpolation on semi-logarithmic paper of the values found for B<sub>1</sub> and B<sub>2</sub>.

#### RESULTS

Values obtained in rats of the Sprague-Dawley strain are presented in table 1, where they are arranged for comparison with estimates of the same and similar renal

functions made by others. The procedure described is at least as satisfactory as any. Reproducibility in successive estimates at intervals of 7 to 10 days in individual rats is shown in table 2. Values are reported per 100 gm. body weight rather than in

TABLE 1. SUMMARY OF OBSERVATIONS ON RENAL FUNCTIONS IN NORMAL RATS

SERIES	FUNCTION	NO. OF OBSERVATIONS	ANIMALS	MEANS	$\sigma$ MEAN	100 $\sigma$ MEAN MEAN
Own	C <sub>CR</sub>	91	39	0.61	.016	2.6
	Tm <sub>PAH</sub>		39	0.29	.0056	1.9
Braun-Menendez and Chiodi	C <sub>Inulin</sub>	84	84	0.60	.031	5.2
	Tm <sub>diodrast-1</sub>	30	30	0.183	.0135	7.4
Friedman, M.	C <sub>CR</sub>	31		0.66	.028	4.3
Friedman, S., Polley and Friedman	C <sub>Inulin</sub>	14	14	0.65	.019	2.9
	Tm <sub>PAH</sub>	14	14	0.18	.005	2.8
Dicker and Heller	C <sub>Inulin</sub>	104	35	0.35	.0027	0.8
	Tm <sub>diodrast-1</sub>			0.126	.0027	2.1
Corcoran and Page	C <sub>mannitol</sub>	29	20	0.55	.085	9.7
	Tm <sub>PAH</sub>	29	20	0.327	.016	4.9

Summary of observations of renal functions in this and other series of observations in normal rats. Data of other authors are recalculated, where necessary, applying corrections for body weight and urine volume to bring them in line with observations in the present series in which body weight averages 229 gm. and urine volume about 0.2-0.4 cc/10 min. Results are expressed as cc. plasma clearance or mg. Tm per 100 gm. body weight per minute. The data of Corcoran and Page were obtained by a method similar to that here described. The greater variability presumably reflects the effects of light anesthesia and inaccuracy due to single blood sampling.

TABLE 2. VARIABILITY OF RENAL FUNCTION DETERMINATIONS

RAT NO.	Tm <sub>PAH</sub> MG/100 GM.							C <sub>CR</sub> CC. PER 100 GM.						
	1	2	3	4	5	6	Mean	1	2	3	4	5	6	Mean
15	0.34	0.27	0.39	0.34			0.33	0.70	0.70	0.75	0.56			0.67
40	0.32	0.24	0.37	0.35	0.29	0.33	0.32	0.63	0.64	0.76	0.68	0.61	0.57	0.64
46	0.32	0.28	0.34	0.25			0.30	0.59	0.68	0.77	0.51			0.63
47	0.26	0.26	0.24	0.31			0.27	0.55	0.71	0.77	0.82			0.63
48	0.25	0.37	0.31	0.28	0.29		0.30	0.47	0.56	0.66	0.47	0.51		0.53
50	0.33	0.29	0.27	0.25			0.28	0.49	0.45	0.38	0.47			0.45
51	0.29	0.36	0.37	0.35			0.34	0.44	0.84	0.80	0.61			0.67
54	0.26	0.29	0.29	0.24			0.27	0.91	0.49	0.48	0.66			0.63
65	0.34	0.36	0.34	0.36			0.35	0.67	0.69	0.67	0.69			0.68
66	0.28	0.24	0.26	0.25			0.26	0.76	0.73	0.69	0.58			0.69

Successive observations of Tm<sub>PAH</sub> and C<sub>CR</sub> in normal rats at intervals of 7 to 10 days.

surface area units because of the simplicity of calculation and because, in the narrow range of body weight present in our group, the surface area units offered no advantage.

#### DISCUSSION

Factors making for accuracy in this method are a) complete collection of urine by bladder washing in contrast to digital expression of urine; b) the increased accu-

racy of collection due to the diuretic effect of mannitol (mean plasma mannitol concentration during the test period is about 150 mg/100 cc.); c) the volumes of blood withdrawn are small, so that the procedure is neither shocking nor depleting, while d) the analyses are done on plasma rather than whole blood which, in our hands, is not as satisfactory as plasma for determinations of PAH and mannitol and is unsuitable for creatinine; e) the brevity of the clearance period and the measurement of two blood samples minimize inaccuracies in the estimate of mean plasma concentration; f) the plasma concentrations obtained average about 50 mg. PAH and 25 mg. creatinine/100 cc.; analyses in normal rats indicate that the curve of plasma concentrations are either level or slowly falling; the concentrations are such as to be accurately measurable in 1/100 plasma filtrate.

The mean creatinine clearance found in our series corresponds well with other estimates of creatinine and inulin clearances. The mean  $Tm_{PAH}$  is greater than that reported by Friedman, Polley and Friedman (5). This is because the plasma concentration maintained in our procedure is such as to saturate the tubular excretory mechanism for PAH. That this is so is indirectly confirmed by the ratio of mean  $Tm_{PAH}$  in our series to mean  $Tm_{DIODRASE-I}$  in the series of Braun-Menendez and Chiodi. The ratio is 1.58, which corresponds with this value in other species. The estimates of glomerular filtration and tubular excretory function respectively from inulin clearance and  $Tm_{DIODRASE-I}$  by Dicker and Heller (2) are low in comparison with our observations and those of others. Insofar as these differences are not procedural, they may be attributed to differences between rat groups found by Corcoran and Page (6.)

#### SUMMARY

A method is described for the measurements of creatinine clearance and  $Tm_{PAH}$  in rats under conditions which favor accuracy and reproducibility. The procedure is adaptable to the bio-assay in rats of substances which affect these renal excretory functions.

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# BUFFER EQUILIBRIA AND REABSORPTION IN THE PRODUCTION OF URINARY ACIDITY

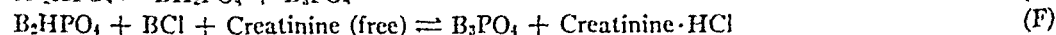
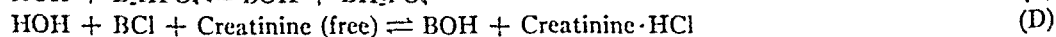
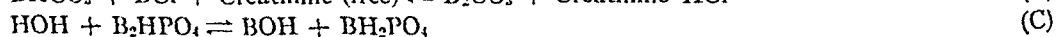
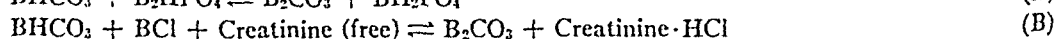
WALTER MENAKER<sup>1</sup>

From the Department of Internal Medicine<sup>2</sup>, Yale University School of Medicine, and the Medical Division, Montefiore Hospital

NEW HAVEN, CONNECTICUT, AND NEW YORK CITY

VARIOUS explanations for the titratable acidity of the urine have been reviewed by Pitts *et al.* (1-3), who postulate *active* transport of acid by the renal tubular cells, either as ionic exchange against a concentration gradient (4) or as frank acid secretion (5), as the mechanism responsible for titratable urinary acidity under all circumstances. They cite their experiments (1, 3) as conclusively demonstrating this mechanism.

The concentrations in glomerular filtrate of  $B_2CO_3$  (about  $10^{-4}$  M),  $BOH$  ( $10^{-7}$  to  $10^{-6}$  M) and  $B_3PO_4$  ( $10^{-8}$  M) are so extremely small that it has been generally held that these compounds can be of no physiological significance. However, since far larger amounts of  $B_2CO_3$ ,  $BOH$  or  $B_3PO_4$  could be reabsorbed from the glomerular filtrate than exist therein at any given moment, these compounds might play important rôles in the production of urinary acidity. So far as these compounds are concerned, the following equilibrium reactions must obtain:



That a definite equilibrium, characterized by the classical constant  $K_{eq}$ , exists for each of these reactions can be shown by deriving a general equation that explicitly describes the equilibrium existing among weak acids and bases and their salts in aqueous solution. Employing the convention of Peters and Van Slyke (6), where  $Ha$  is a weak acid,  $boh$  a weak base,  $HA$  a strong acid, and  $BOH$  a strong base:



where

$$K_{eq} = \frac{[Ba_1][Ha_{11}]}{[Ha_1][Ba_{11}]}$$

according to the equation of Henderson (7, 8):

$$[H^+] = K_{a1} \frac{[Ha_1]}{[Ba_1]} = K_{a11} \frac{[Ha_{11}]}{[Ba_{11}]}$$

Received for publication May 24, 1948.

<sup>1</sup> Dazian Foundation Medical Research Fellow.

<sup>2</sup> Present address, 83<sup>rd</sup> Fairmount Place, New York City.

After transposing:

$$\frac{[Ba_1][Ha_{11}]}{[Ha_1][Ba_{11}]} = \frac{Ka_1}{Ka_{11}}$$

$$K_{eq} = \frac{Ka_1}{Ka_{11}} = \text{antilog}(pKa_{11} - pKa_1), \text{ or } pK_{eq} = pKa_1 - pKa_{11}.$$



$$K_{eq} = \frac{Kb_{11}}{Kb_1} = \text{antilog}(pKb_1 - pKb_{11}), \text{ or } pK_{eq} = pKb_{11} - pKb_1.$$



where BA, like HOH, is sufficiently large to remain rather constant,

$$K_{eq} = \frac{Kab_1}{Kab_{11}} = \text{antilog}(pKab_{11} - pKab_1), \text{ or } pK_{eq} = pKab_1 - pKab_{11}.$$

This equation also applies to (I) and (II).  $p$  is the negative logarithm of any value.  $Kab$  of an acid  $Ha$  equals its  $Ka$ .  $Kab$  of a base  $bOH$  equals  $\frac{K_w}{K_b}$  where  $K_w = [H^+][OH^-] = 10^{-14}$  at  $25^\circ$  C. and  $10^{-13.6}$  at  $38^\circ$  C.  $Kab$  of an acid or base equals the  $H^+$  ion concentration at half-neutralization of the acid or base. For (I) and (II) as well as (III), where the subscript<sub>1</sub> is assigned to the weak electrolyte whose more acid component of its buffer pair is on the left side of the equation, the point of equilibrium is defined by the equation:

$$pK_{eq} = pKab_1 - pKab_{11}. \quad (G)$$

Reactions (A), (C), and (E) are examples of (I), and (B), (D), and (F) are examples of (III). In actual practice,  $pK$  values are used. For example, as  $pK'$  of  $BHCO_3$  is 9.8 and that of  $BH_2PO_4$  is 6.8, tubular reabsorption of  $B_2CO_3$  would drive reaction (A) to the right to keep  $pK_{eq} = 9.8 - 6.8$  (i.e., to maintain  $K_{eq}$  at a value of  $10^{-3}$ ). The same process would move (B) to the right to keep  $pK_{eq}$  of this equation at  $9.8 - 4.7$  ( $K_{eq} = 8 \times 10^{-6}$ ). Consequently  $B_2CO_3$  would be constantly reformed while it was being reabsorbed. Similarly reabsorption of  $BOH$  would move (C) and (D) to the right; and reabsorption of  $B_3PO_4$  would move (E) and (F) to the right.

If tubular reabsorption can fully account for urinary acidity, the urine, from the standpoint of buffer content and pH, is actually glomerular filtrate minus the reabsorbate. If this is the case, the solution resulting from the addition of reabsorbate to fully elaborated urine should be identical with the glomerular filtrate with respect to its buffer composition and pH. To test the validity of this hypothesis, glomerular filtrates and their corresponding urines were prepared to conform to published analyses (1). To the urines were added reabsorbates calculated to conform to these data. The resulting solutions proved to be identical with the original glomerular filtrates in buffer composition and pH. Presumably this procedure restored to the urine what the kidney had previously removed by reabsorption. Since the calculated reabsorbates contained significant amounts of carbonate, the titratable acidities of the urines under discussion may, therefore, be fully accounted for by



tubular reabsorption of carbonate together with bicarbonate without the necessity of postulating either tubular secretion of acid or exchange in the tubules of  $H^+$  for  $B^+$  ions. Since the lumen of the renal tubule is extracellular, any buffer equilibria or any relationship between urine, reabsorbate, and glomerular filtrate that can be demonstrated *in vitro* can be expected to exist in the colloid-free, aqueous filtrate in the tubular lumen.

If  $B_2HPO_4$  and  $BH_2PO_4$  are reabsorbed in the ratio in which they occur in the original glomerular filtrate (isohydric reabsorption), such reabsorption does not contribute to titratable urinary acidity. The less phosphate there is in the remaining tubular urine, the poorer is its buffering power, and hence the more markedly is its pH lowered by  $BHCO_3$  or  $B_2CO_3$  reabsorption.

#### METHODS

The experiment was performed in U-tubes with sufficient mercury in the bottom to keep a solution in one arm completely separate from a solution in the other arm when the tube was in an upright (U) position. By this means the alkaline reabsorbate containing carbonate and bicarbonate could be prepared in one arm of the tube, while the acid urine was prepared in the other. The total amount of water used was equal to that contained in urine plus reabsorbate, approximately equal volumes being introduced into each arm. Equal amounts of phenol red indicator were added to the prepared solutions and the colorimetric standards. When necessary, additional mercury was added to bring the total volume near the full capacity of the U-tube. After all visible air-bubbles had been removed, a small supplement of oil (about 1.5 cc. in each arm) was added to permit the insertion of rubber stoppers without the loss of any of the aqueous solution. No visible air was allowed to remain in the U-tube. With the tube properly stoppered, it was carefully inverted and the contents judiciously mixed (avoiding emulsification of the oil) until there resulted a uniform color, which was compared with known colorimetric standards (Sørensen phosphate buffers with phenol red indicator in U-tubes of the same dimensions) 0.05 pH apart from each other.

The glomerular filtrate was likewise prepared in a U-tube. The carbonate and bicarbonate were kept in one arm, separated by mercury from the other arm containing more acidic material. After the tube was properly sealed and mixed (with phenol red indicator included), the pH of the resulting 'glomerular filtrate' solution was compared with the known colorimetric standards and with the 'urine plus reabsorbate' solution.

Since bicarbonate solutions rapidly lose  $CO_2$  to the air, sodium carbonate was used with an equimolar amount of HCl. However, in the phosphate experiment, bicarbonate was also used, as an alternate procedure, in preparing the glomerular filtrate and, in conjunction with carbonate, in preparing the reabsorbate. (See under B of table 1.) In preparing the glomerular filtrate, carbonate was introduced into one arm, while the HCl was placed in the other. In preparing the reabsorbate, however, it was possible to put both in the same arm without significant loss of  $CO_2$  if the HCl and buffer substances were added first, followed by the rapid addition of  $Na_2CO_3$  along the wall of the tube. The thick layer of  $Na_2CO_3$ , over the acid-carbonate inter-

face where  $\text{H}_2\text{CO}_3$  is formed, reacts with any rising  $\text{CO}_2$  or  $\text{H}_2\text{CO}_3$  to form  $\text{NaHCO}_3$ . (But acid layered over  $\text{Na}_2\text{CO}_3$  will not prevent the escape of  $\text{CO}_2$ .) The pH of the prepared 'urines' was determined electrometrically. Accuracy in the amounts of  $\text{B}_2\text{CO}_3$  and  $\text{HCl}$  used is exceedingly important, since these compounds affect pH most markedly, especially in the creatinine experiment. Sufficient sodium chloride

TABLE 1. PHOSPHATE EXPERIMENT (FIGURES ARE IN MM/MIN.)

*Buffer and salt content, and materials used in preparation, of urine, reabsorbate, and glomerular filtrate*

	TOTAL CREATININE	CREATININE (FREE)	CREATININE · HCl	B <sub>2</sub> Cl	TOTAL Cl	H <sub>2</sub> CO <sub>3</sub>	BHCO <sub>3</sub>	B <sub>2</sub> CO <sub>3</sub>	TOTAL CO <sub>2</sub>	BH <sub>2</sub> PO <sub>4</sub>	B <sub>2</sub> HPO <sub>4</sub>	TOTAL PO <sub>4</sub>	TOTAL B	HCl
<b>A</b>														
1. Filtrate														
a) Calculated	0.171	0.171	0.000	7.516	7.516	0.055	1.304	0.008	1.367	0.102	0.510	0.612	9.958	
b) Ingredients		0.171		6.000				1.367			0.612			1.516
2. Urine														
a) Calculated	0.171	0.159	0.012	0.400	0.412	(0.0)	(0.0)	(0.0)	(0.0)	0.400	0.089	0.489	0.978	
b) Ingredients		0.171		0.000				0.000			0.489			0.412
3. Reabsorbate														
a) Calculated	0.000	0.000	0.000	7.104	7.104	0.000	1.104	0.263	1.367	0.000	0.123	0.123	8.980	
b) Calculated	0.000	0.000	0.000	7.104	7.104	0.000	1.084	0.283	1.367	0.020	0.103	0.123	8.980	
c) Ingredients		0.000		6.000				1.367			0.123			1.104
4. U + R: 'Totals'	0.171				7.516				1.367			0.612	9.958	
5. U + R: Ingredients		0.171		6.000				1.367			0.612			1.516
<b>B</b>														
1. Filtrate														
a) Ingredients	0.171	0.171		7.367	7.516		1.367	0.000	1.367		0.612	0.612	9.958	0.149
2. Reabsorbate														
a) Ingredients	0.000	0.000		7.104	7.104		1.104	0.263	1.367		0.123	0.123	8.980	0.000
3. Urine														
a) Ingredients	0.171	0.171		0.000	0.412		0.000	0.000	(0.0)		0.489	0.489	0.978	0.412
<b>C</b>														
1. Reabsorbate: 0.283 BOH	0.000			7.104	7.104		1.367		1.367	0.020	0.103	0.123	8.980	
2. Reabsorbate: 0.123 B <sub>2</sub> PO <sub>4</sub>	0.000			7.104	7.104		1.227	0.140	1.367			0.123	8.980	

NOTES: 'Ingredients' are the source materials used. 'Calculated' content refers to probable distribution of compounds at the pH of the solution. 'U + R' is urine plus reabsorbate. 'Totals' means the totals of the various moieties. In A, reabsorbate a) has  $\text{B}_2\text{HPO}_4$  only, while b) assumes isohydric  $\text{PO}_4$  reabsorption. In B,  $\text{BHCO}_3$  is one of the ingredients used in glomerular filtrate and in reabsorbate. In C, 1 is a reabsorbate containing 0.283 BOH, and 2 is one containing 0.123  $\text{B}_2\text{PO}_4$ . 'Filtrate' is 73.1 cc/min. of glomerular filtrate with pH 7.34 at 38°C. or 7.55 at 22°C. 7.6 cc/min. of urine with pH 6.06 at 22°C. has titratable acidity to pH 7.35 of 0.312 mEq/min. 65.5 cc/min. of reabsorbate has calculated pH of 9.2. Source of essential data is first line of Table 1 of Pitts and Alexander (1).

was added to approximate the chloride concentration of the glomerular filtrate usually encountered in mammals.

The  $\text{pK}'_{\text{ab}}$  of creatinine, little affected by ionic strength, shifts markedly as temperature changes ( $\text{pK}'_{\text{ab}}$  is 5.0 at 22° and 4.7 at 38°C.).  $\text{BH}_2\text{PO}_4$  has a  $\text{pK}'$  which is markedly affected by ionic strength or concentration ( $\text{pK}'$  is 6.8 at ionic strength 0.16) but declines only 0.03 pH as temperature rises from 20° to 38°C.

Data for plasma pH, as reported by Pitts (1), had been obtained by subtracting 0.014 pH per degree difference between room temperature and body temperature (38°C.); the actual pH measurement had been made at room temperature. Since the present solutions were prepared at  $22 \pm 2^\circ\text{C}$ ., they were made to have a pH 0.2 higher than Pitts' published values (1) which were for 38°C.

The titratable acidity of the prepared 'urine' was determined by titrating it to pH 7.35 (determined electrometrically), the end-point used in previous work.

Calculation of reabsorbates was carried out as follows: In the phosphate experiment (see table 1), the glomerular filtrate of pH 7.55 at room temperature (7.34 at 38°C.) had a total  $\text{PO}_4$  of 0.612; total creatinine 0.171; total  $\text{CO}_2$  1.367; an assumed total chloride of 7.516, obtained by including NaCl to approximate a normal chloride content; and total B of 9.958, part of it due to the NaCl included.

Urine of pH 6.06 at room temperature had total creatinine of 0.171; total phosphate 0.489; negligible total  $\text{CO}_2$ , Pitts having made no attempt to retain or measure  $\text{CO}_2$ ; an assumed total Cl of 0.410, obtained by including a source of BCl, which helped bring total B to 0.978. Figures are in terms of mM/min.

The reabsorbate, being glomerular filtrate minus urine, therefore contains:

$$\text{Total B} = 9.958 - 0.978 = 8.980$$

$$\text{Total P} = 0.612 - 0.489 = 0.123$$

$$\text{Total CO}_2 = 1.367 - 0.0 = 1.367$$

$$\text{Total creatinine} = 0.171 - 0.171 = 0.000$$

$$\text{Total Cl} = 7.516 - 0.412 = 7.104.$$

Hence, in the reabsorbate, of total B (8.980), 7.104 is BCl, as there is 7.104 Cl. The remaining B,  $8.980 - 7.104 = 1.876$ , may be divided among  $\text{B}_2\text{HPO}_4$ ,  $\text{BHCO}_3$ , and  $\text{B}_2\text{CO}_3$ . If the total P is assumed to be in the form of  $\text{B}_2\text{HPO}_4$ , there will be 0.123  $\text{B}_2\text{HPO}_4$ , accounting for 0.246 B. The remaining B,  $1.876 - 0.246 = 1.630$ , is divided between  $\text{BHCO}_3$  and  $\text{B}_2\text{CO}_3$ . As total  $\text{CO}_2$  is 1.367, there is  $1.630 - 1.367 = 0.263$  more B than  $\text{CO}_2$ . Thus there are 0.263  $\text{B}_2\text{CO}_3$  and  $1.367 - 0.263 = 1.104$   $\text{BHCO}_3$  in the reabsorbate. This and alternate reabsorbates, including two where isohydric phosphate reabsorption is assumed, are listed in table 1. Similarly calculated reabsorbates for the creatinine are listed in table 2. Any NaCl added does not affect carbonate and bicarbonate content of the reabsorbate.

## RESULTS

The data presented in table 1 describe the pH and buffer content of the glomerular filtrate and urine. This is patterned after the pH and buffer content reported on the first line of table 1 by Pitts and Alexander (1) and based on the amounts of such substances needed to prepare such solutions. The data presented in table 2 do likewise with the glomerular filtrate and urine described on the first line of table 2 of the same paper.

The present tables (1 and 2) include the calculated reabsorbates and the materials that can be used in preparing the urines, reabsorbates, and glomerular filtrates. It may be noted that the total  $\text{CO}_2$ , total phosphate, etc., of the 'urine plus reabsorbate' equal the total for each of these moieties in the 'glomerular filtrate'. Furthermore, these tables show that when  $\text{Na}_2\text{CO}_3$ , HCl,  $\text{Na}_2\text{HPO}_4$ , creatinine, and NaCl are used as source materials, the amount of each of these used for the urine plus the amount of each used for the reabsorbate equals the amount of each used for the glomerular filtrate.

Colorimetric pH determinations showed that the 'urine plus reabsorbate' had the same pH as the corresponding 'glomerular filtrate'. This pH was  $7.55 \pm 0.03$  at room temperature, to correspond to pH 7.34 at 38°C., for the phosphate experiment

(table 1) and  $7.45 \pm 0.03$  at room temperature, corresponding to pH 7.25 at  $38^\circ\text{C}$ ., for the creatinine experiment (table 2). Room temperature was  $22 \pm 2^\circ\text{C}$ . An error due to the inclusion of 10 per cent more  $\text{Na}_2\text{CO}_3$  at the expense of  $\text{NaHCO}_3$  (keeping total  $\text{CO}_2$  constant) would raise the pH of the 'urine plus reabsorbate' 0.09 in the phosphate experiment and about 0.3 in the creatinine experiment. This indicates that the experimental data here presented are correct to within  $\pm 3$  or 4 per cent for the phosphate experiment and within  $\pm 2$  per cent for the creatinine experiment.

TABLE 2. CREATININE EXPERIMENT (FIGURES ARE IN MM/MIN.)

*Buffer and salt content, and materials used in preparation, of urine, reabsorbate, and glomerular filtrate*

	TOTAL CREATININE	CREATININE (FREE)	CREATININE · HCl	BCl	TOTAL Cl	H <sub>2</sub> CO <sub>3</sub>	BHCO <sub>3</sub>	B <sub>2</sub> CO <sub>3</sub>	TOTAL CO <sub>2</sub>	BH <sub>2</sub> PO <sub>4</sub>	B <sub>2</sub> HPO <sub>4</sub>	TOTAL PO <sub>4</sub>	TOTAL B	HCl
<i>A</i>														
1. Filtrate:														
a) Calculated.....	1.250	1.247	0.003	9.425	9.428	0.022	0.518	0.002	0.542	0.003	0.015	0.018	9.980	
b) Ingredients.....		1.250		8.860				0.542			0.018			0.568
2. Urine:														
a) Calculated.....	1.250	1.103	0.147	2.601	2.748	(0.0)	(0.0)	(0.0)	(0.0)	0.001	0.000	0.001	2.602	
b) Ingredients.....		1.250		2.600				0.000			0.001			0.148
3. Reabsorbate:														
a) Calculated.....	0.000	0.000	0.000	6.680	6.680	0.000	0.420	0.122	0.542	0.000	0.017	0.017	7.378	
b) Calculated.....	0.000	0.000	0.000	6.680	6.680	0.000	0.417	0.125	0.542	0.003	0.014	0.017	7.378	
c) Ingredients.....		0.000		6.260				0.542			0.017			0.420
4. U + R: 'Totals'.....	1.250				9.428				0.542					
5. U + R: Ingredients.....		1.250		8.860				0.542			0.018	0.018	9.980	0.568
<i>B</i>														
1. Reabsorbate: 0.125 BOH.....	0.000			6.680	6.680		0.542		0.542	0.003	0.014	0.017	7.378	
2. Reabsorbate: 0.017 B <sub>2</sub> PO <sub>4</sub> .....	0.000			6.680	6.680		0.437	0.105	0.542			0.017	7.378	

NOTES: 'Ingredients' are the source materials used. 'Calculated' content refers to the probable distribution of compounds at the pH of the solution. 'U + R' is urine plus reabsorbate. 'Totals' means the totals of the various moieties. In A, reabsorbate a) has  $\text{B}_2\text{HPO}_4$  only while b) assumes isohydric  $\text{PO}_4$  reabsorption. 'Filtrate' is 78.7 cc/min. of glomerular filtrate with pH 7.25 at  $38^\circ\text{C}$ . or 7.45 at  $22^\circ\text{C}$ . 16.5 cc/min. of urine with pH 5.84 at  $22^\circ\text{C}$ . has titratable acidity to pH 7.35 of 0.144 mEq/min. 62.2 cc/min. of reabsorbate has calculated pH of 9.25. In B, 1) is a reabsorbate containing 0.125 BOH, and 2) is one containing 0.017  $\text{B}_2\text{PO}_4$ . Source of essential data is first line of Table 2 of Pitts and Alexander (1).

The titratable acidity of the urine in table 1 (phosphate experiment) is 0.312 mEq/min. to pH 7.35, while that of the urine in table 2 (creatinine experiment) is 0.144 mEq. to pH 7.35.  $\text{BHCO}_3$  plus  $\text{B}_2\text{HPO}_4$  reabsorption could, according to Pitts, account for only 33 per cent of the titratable acidity of the urine in table 1 and for only 25 per cent in table 2. Therefore, although there is 3 or 4 times as much  $\text{BHCO}_3$  as  $\text{B}_2\text{CO}_3$  in the calculated reabsorbate, reabsorption of  $\text{B}_2\text{CO}_3$  will account for 2 or 3 times as much of the titratable urinary acidity as will reabsorption of  $\text{BHCO}_3 + \text{B}_2\text{HPO}_4$ , i.e., 67 per cent in one case and 75 per cent in the other. To account for any titratable acidity reported (1, 3), less than 25 per cent of the total  $\text{CO}_2$  reabsorbed need be  $\text{B}_2\text{CO}_3$ , over 75 per cent being  $\text{BHCO}_3$ .

## DISCUSSION

Since the pH and the totals of the moieties in the 'urine plus reabsorbate' in tables 1 and 2 are identical with those in the 'glomerular filtrate', the experimental

procedure may be regarded as reversing the reabsorptive processes of the kidney. The results demonstrate that the published data on titratable acidity (1, 3), assuming

TABLE 3. ARTIFICIAL STEP-BY-STEP DESCRIPTION OF CONVERSION OF BUFFER (AND CHLORIDE) CONTENT OF GLOMERULAR FILTRATE TO THAT OF URINE IN PHOSPHATE EXPERIMENT  
(FIGURES ARE IN MM/MIN.)

Reaction: 1. 2. 3. 4.	BCl + BCl	H <sub>2</sub> CO <sub>3</sub> + H <sub>2</sub> CO <sub>3</sub> + Creatinine	Creatinine +	= B <sub>2</sub> HPO <sub>4</sub> B <sub>2</sub> HPO <sub>4</sub> +	BHCO <sub>3</sub> = BHCO <sub>3</sub> + BHCO <sub>3</sub> BHCO <sub>3</sub>	+ + BH <sub>2</sub> PO <sub>4</sub> = BH <sub>2</sub> PO <sub>4</sub> =	Creat·HCl + Creat·HCl	B <sub>2</sub> CO <sub>3</sub> + B <sub>2</sub> CO <sub>3</sub>
Steps: 'G. F.' A	7.516	0.055	0.171	0.510	1.304	0.102	0.000 4 × 10 <sup>-4</sup>	0.008
B	R7.104			Ro.103		Ro.020		
C	0.412	0.055	0.171	0.407	1.304	0.082	0.000	0.008
D	0.412	0.055	0.171	-0.275	-0.275	+0.275		+0.275
E	-0.001		-0.001	0.132	1.029	0.357	0.000	0.283
F	0.411	0.055	0.170	0.132	1.028	0.357	+0.001	+0.001
G	0.411	0.055	0.171	0.132	1.028	0.357	0.001	0.284
H	0.411	0.055	0.171	0.132	1.028	0.357	0.001	Ro.283
I		+0.118		+0.118	-0.118	-0.118		0.001
J	0.411	0.173	0.170	0.250	0.910	0.239	0.001	0.001
K					Ro.910			1 × 10 <sup>-1</sup>
L	0.411	0.173	0.170	0.250	0.000	0.239	0.001	0.001
M	-0.001	-0.011	-0.011		+0.011		+0.011	
N	0.400	0.162	0.159	0.250	0.011	0.239	0.012	0.001
O		-0.162		-0.162	+0.162	+0.162		
P	0.400	0.000	0.159	0.088	0.173	0.401	0.012	0.001
Q					Ro.173			
R	0.400	0.000	0.159	0.088	0.000	0.401	0.012	0.001
S				+0.001	+0.001	-0.001		-0.001
T	0.400	0.000	0.159	0.089	0.001	0.400	0.012	0.000
U					Ro.001			
Urine	0.400	0.000	0.159	0.089	0.000	0.400	0.012	0.000
Total reabsorbed	7.104			0.103	1.084	0.020		0.283

STEPS: A. Composition at room temperature of original glomerular filtrate of pH 7.55 (pH 7.34 at 38°C.). B. Reabsorption of 7.104 mM NaCl and isohydric reabsorption of 0.123 mM phosphate. C, D and E. Reactions 3 and 4 are moved to the right due to reabsorption of 0.283 mM B<sub>2</sub>CO<sub>3</sub> even as it is reformed. F. Reaction 2 is moved to the left by the excess BH<sub>2</sub>PO<sub>4</sub> and BHCO<sub>3</sub>, reaching equilibrium at pH 6.8 at body temperature. G, H, J and K. Reactions 1 and 2 are moved to the right due to BHCO<sub>3</sub> being reabsorbed even as it is reformed. L and M. The excess B<sub>2</sub>CO<sub>3</sub> moves 3 to the left, reaching equilibrium at pH 6.06 at room temperature after all BHCO<sub>3</sub> is reabsorbed. This is 7.60 cc. of urine. (R = reabsorb.) Glomerular filtrate is 73.1 cc.

isohydric phosphate reabsorption, can be fully explained by tubular reabsorption of B<sub>2</sub>CO<sub>3</sub> along with BHCO<sub>3</sub> without the necessity of tubular secretion of acid or ionic exchange.

Tables 3 and 4 show in artificial step-by-step fashion how the buffer (and salt)

content of the glomerular filtrate of the phosphate and creatinine experiments (respectively) is converted to that of the urine. These tables show that if reabsorption of  $B_2CO_3$  preceded  $BHCO_3$  reabsorption but follow isohydric reabsorption of phosphate, the concentration of  $B_2CO_3$  in the remaining tubular urine would, at

TABLE 4. ARTIFICIAL STEP-BY-STEP DESCRIPTION OF CONVERSION OF BUFFER (AND CHLORIDE) CONTENT OF GLOMERULAR FILTRATE TO THAT OF URINE IN CREATININE EXPERIMENT  
(FIGURES ARE IN MM/MIN.)

Reaction: 1. 2. 3. 4.	BCl+	$H_2CO_3 +$ $H_2CO_3$	Creatinine +	= $B_2HPO_4$ $B_2HPO_4$ +	$BHCO_3$ = $BHCO_3$ + $BHCO_3$ $BHCO_3$	+ $+BH_2PO_4$ = $BH_2PO_4$ =	Creat·HCl + Creat·HCl	$B_2CO_3$ + $B_2CO_3$
BCl		+	Creatinine					
Steps:								
'G.F.' A	9.425	0.022	1.247	0.015	0.518	0.003	0.003	0.002
B	R6.680			Ro.014		Ro.003		
	2.745	0.022	1.247	0.001	0.518	0.000	0.003	0.002
						$2 \times 10^{-4}$		
C	-0.122		-0.122		-0.122		+0.122	+0.122
	2.623	0.022	1.125	0.001	0.396	0.000	0.125	0.124
D				-0.001	-0.001	+0.001		+0.001
	2.623	0.022	1.125	0.000	0.395	0.001	0.125	0.125
E								Ro.125
	2.623	0.022	1.125	0.000	0.395	0.001	0.125	0.000
F	+0.105	+0.105	+0.105		-0.105		-0.105	
	2.728	0.127	1.230	0.000	0.290	0.001	0.020	0.000
				$3 \times 10^{-4}$		$7 \times 10^{-4}$		$1.4 \times 10^{-4}$
G					Ro.290			
	2.728	0.127	1.230	0.000	0.000	0.001	0.020	0.000
H	-0.127	-0.127	-0.127		+0.127		+0.127	
	2.601	0.000	1.103	0.000	0.127	0.001	0.147	0.000
J					Ro.127			
Urine:	2.601	0.000	1.103	0.000	0.000	0.001	0.147	0.000
				$1 \times 10^{-4}$		$9 \times 10^{-4}$		
Total reabsorbed	6.680			0.014	0.417	0.003		0.125

STEPS: A. Composition at room temperature of original glomerular filtrate of pH 7.45 (pH 7.25 at 38°C.). B. Reabsorption of 6.680 mM NaCl and isohydric reabsorption of 0.017 mM phosphate. C, D and E. Reactions 3 and 4 are moved to the right due to reabsorption of 0.125 mM  $B_2CO_3$  even as it is reformed. F. Reaction 1 is moved to the left by the excess  $BHCO_3$  and creatinine·HCl; equilibrium is reached at pH 6.5 (at 38°C.). G, H and J. Reaction 1 and 2 are moved to the right due to  $BHCO_3$  being reabsorbed, even as it is reformed, the result being 16.5 cc. of urine at pH 5.84 at room temperature (R = reabsorb.) Glomerular filtrate is 78.7 cc.

the end of  $B_2CO_3$  reabsorption, be  $0.7 \times 10^{-4}$  Molar (assuming 15 cc. of urine remains) at pH 6.8 in the phosphate experiment and  $0.7 \times 10^{-5}$  Molar (assuming 20 cc. of urine remains) at pH 6.5 in the creatinine experiment. If reabsorption of  $B_2CO_3$  and  $BHCO_3$  began together and proceeded at the same rate (but followed isohydric phosphate reabsorption), the concentration of  $B_2CO_3$  in the tubular urine at the completion of  $B_2CO_3$  reabsorption would be  $0.5 \times 10^{-4}$  Molar (at pH 6.75 in 12 cc. of urine remaining) in the phosphate experiment and  $0.3 \times 10^{-5}$  Molar (at pH 6.3 in

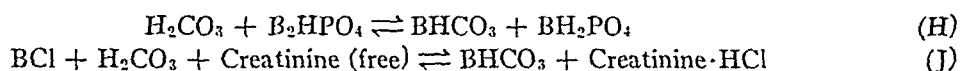
20 cc. of urine remaining) in the creatinine experiment. Since  $10^{-5}$  Molar is the approximate concentration of  $\text{BHCO}_3$  in urines of low pH, there should be no more difficulty in accepting the view that reabsorption of  $\text{B}_2\text{CO}_3$  can occur than in accepting the widely held view that reabsorption of  $\text{BHCO}_3$  (8, 14) can occur. Indeed these concentrations ( $0.7 \times 10^{-5}$  to  $0.7 \times 10^{-4}$  Molar) of  $\text{B}_2\text{CO}_3$  are about 200 to 2000 times the concentration in blood of the  $\text{H}^+$  ion (about  $0.4 \times 10^{-7}$  Molar), which is involved in the ionic exchange. (These statements also apply to the urines with the highest titratable acidity and lowest pH reported (1, 3) by Pitts.) Hence, if probability parallels concentration, reabsorption of  $\text{B}_2\text{CO}_3$  (with  $\text{BHCO}_3$ ) is, on the basis of known concentrations, a more probable explanation of the titratable urinary acidity than is ionic exchange.

Ionic exchange of  $\text{IB}^+$  for  $\text{IH}^+$  ion has virtually the same effect on the buffer content and pH of the tubular cell and of the tubular urine as the reabsorption of 1 molecule of  $\text{BOH}$ . The resulting formation of considerable  $\text{H}_2\text{CO}_3$  or  $\text{CO}_2$  in the tubular urine has not been mentioned in any of the diagrams or discussions (1, 2, 3, 9) of the acidification of the urine by ionic exchange, which is, therefore, not so simple and direct a process as appears at first sight. The  $\text{H}_2\text{CO}_3$  or  $\text{CO}_2$  thus formed is presumed (4) to diffuse slowly into the tubular cells. This is tantamount to the extreme separation of the transferred materials into  $\text{BOH} + \text{H}_2\text{CO}_3$  or  $2\text{BOH} + \text{H}_2\text{CO}_3$  (instead of  $\text{BHCO}_3$  or  $\text{B}_2\text{CO}_3$ ). Since the presumed diffusion of  $\text{H}_2\text{CO}_3$  or  $\text{CO}_2$  out of the tubular urine often lags behind ionic exchange, this mechanism would have a more jarring effect on tubular cell pH than reabsorption of an equivalent amount of  $\text{BHCO}_3$  or  $\text{B}_2\text{CO}_3$ .

Theoretically, reabsorption of 1 molecule each of  $\text{BOH} + \text{BHCO}_3$  could, as shown in tables 1 and 2, have the same effect as the reabsorption of 1 molecule of  $\text{B}_2\text{CO}_3$ . This seems a less likely process, however, for several reasons: *a*) the concentration of  $\text{B}_2\text{CO}_3$  in the filtrate far exceeds that of  $\text{BOH}$ ; *b*) the heat content and free energy content of  $\text{B}_2\text{CO}_3 + \text{H}_2\text{O}$  are considerably less than those of  $\text{BOH} + \text{BHCO}_3$ ; *c*) the minimum osmotic work required for the reabsorption of  $\text{B}_2\text{CO}_3 + \text{H}_2\text{O}$  at the pH of the tubular contents when this process occurs is less than that required for the reabsorption (transfer) of  $\text{BOH} + \text{BHCO}_3$ . Reabsorption of  $\text{B}_3\text{PO}_4 + \text{BHCO}_3$  could not fully account for the urinary acidity in these experiments because of the small amount of phosphate reabsorbed. Where infusions of phosphate or creatinine have not been employed, reabsorption of  $\text{BHCO}_3$  (carbonic acid filtration theory) can fully explain urinary acidity (3).

Reabsorption of  $\text{B}_2\text{CO}_3$  might be compared with the ionic exchange mechanism on the basis of probability. Within cells, the concept of ionic exchange can be accepted without serious objections. Similar exchanges, against a concentration gradient, between cellular contents and tubular fluid that is exteriorized present conceptual difficulties. This, of course, is no insurmountable obstacle. From the standpoint of chemical kinetics and thermodynamics, however, reabsorption of  $\text{B}_2\text{CO}_3$  would have an advantage in economy and would seem more probable, because the concentration of this salt in the glomerular filtrate, though small, is enormously greater than that of  $\text{H}^+$  ion in the plasma and because the minimum osmotic work required for the reabsorption (transfer) of  $\text{B}_2\text{CO}_3$  is less than that required for the transfer of  $\text{H}^+$  ions in ionic exchange or in the secretion of molecular acid.

The observation (10) that very high concentrations (tensions) of  $\text{CO}_2$  can be attained in the urine has been interpreted by some as evidence of the impermeability of the tubules to ready diffusion of  $\text{CO}_2$ . If diffusion of  $\text{CO}_2$  occurred, it would be in both directions across the luminal membrane, the net direction and rate varying with circumstances. Any  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) diffusing into the tubular urine would enter into the equilibrium reactions:



Pitts (1) considers the tubules readily permeable to the diffusion of  $\text{CO}_2$ . This assumption renders unnecessary his postulation of *active* transport of acid, such as secretion of acid or ionic exchange against a concentration gradient, because if the  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) concentration of the tubular urine fell below that of the surrounding peritubular fluids and carbonic anhydrase-rich tubular cells,  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) would *diffuse* into the urine. Consequently, reabsorption of  $\text{BHCO}_3$ , as postulated by Peters and Van Slyke (6) and by Sendroy, Seelig, and Van Slyke (11), could fully explain Pitts' experimental results (carbonic acid filtration-diffusion theory). The only active renal function here would be reabsorption of  $\text{BHCO}_3$ . However, if the tubules were not sufficiently permeable to the ready diffusion of  $\text{CO}_2$ , enough  $\text{BHCO}_3$  could not be formed therefrom (by buffer equilibria) and reabsorbed within a given period to produce the titratable acidities of Pitts' experiments. Regardless of the degree of tubular permeability to  $\text{CO}_2$ , the reabsorption of  $\text{B}_2\text{CO}_3$  could occur.

Indirect evidence, adduced in favor of active transport of acid, also favors active transport (reabsorption) of  $\text{BHCO}_3$  and  $\text{B}_2\text{CO}_3$ . The effect of sulfanilamide in decreasing urinary acidity (1, 3, 12) may be attributed to its effect on carbonic anhydrase and various enzyme systems involved in  $\text{BHCO}_3$  and  $\text{B}_2\text{CO}_3$  reabsorption. Calculations of published data (12) show that  $\text{BHCO}_3$  reabsorption is decreased by sulfanilamide. The same may be true of  $\text{B}_2\text{CO}_3$  reabsorption. Furthermore, if  $\text{B}_2\text{CO}_3$  is reabsorbed, its subsequent reaction with  $\text{H}_2\text{CO}_3$  could be slowed owing to a decreased rate of  $\text{H}_2\text{CO}_3$  formation from  $\text{CO}_2$  during inhibition of carbonic anhydrase activity by sulfanilamide. An accumulation of reabsorbed  $\text{B}_2\text{CO}_3$  would cause an abnormal rise in cellular pH, which could impair certain reabsorptive activities. Davenport (13) has withdrawn his hypothesis that carbonic anhydrase is of significance in the secretion of gastric acid. Its function in the kidney is not definitely known.

The hypothesis (1, 3, 14) that the ability of the kidney to excrete acid is determined by its ability to transfer  $\text{H}^+$  ions against a concentration gradient, is weakened considerably by the fact that more acid was eliminated both by man (3) and the dog (1) in urines with a pH of 5.54 and 5.61 respectively, representing a  $\text{H}^+$  ion concentration of about 70 to 1 (as compared to serum pH), than in urines of much lower pH, e.g. 4.5, representing a gradient of 800 to 1. The urines containing more titratable acid had more buffer than the urines of lower pH. The greater titratable acidity observed (1, 3, 9, 14) when more buffer is present or when a buffer with a higher  $\text{pK}_{\text{ab}}$  is infused can be readily explained by the fact that, under these circumstances, more  $\text{BHCO}_3$  and  $\text{B}_2\text{CO}_3$  can be reabsorbed before their concentrations fall to any given level.



The evidence of Conway *et al* (15) that the proximal tubule is impermeable to sodium does not support the view (1) that four fifths of the filtered  $\text{NaHCO}_3$  and  $\text{H}_2\text{CO}_3$  undergo isohydric reabsorption in the proximal tubule. Furthermore, up to 30 per cent of the filtered water appeared in the urine in the experiments on urinary acidification (1).

#### SUMMARY

Experiments are presented to show that because of buffer equilibria, for which an equation is derived, sufficient  $\text{B}_2\text{CO}_3$  can be reabsorbed in addition to  $\text{BHCO}_3$  to produce any titratable urinary acidity yet reported. Physico-chemical and other considerations favoring such a mechanism are discussed. Conditions under which active tubular reabsorption of  $\text{BHCO}_3$  can fully account for titratable urinary acidity are also noted.

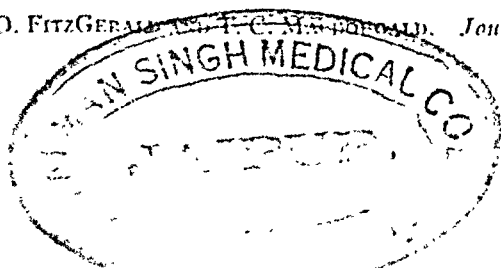
High titratable acidity of the urine can be completely explained as well 1) by active tubular reabsorption of alkaline compounds whether or not the tubular luminal membrane is permeable to the diffusion of  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ), as 2) by active tubular transport of acid (either ionic exchange or secretion of acid) into the tubular lumen followed by passage of  $\text{CO}_2$  ( $\text{H}_2\text{CO}_3$ ) out of the tubular lumen, or 3) by some combination of 1) and 2).

The mechanism responsible for the production of urinary acidity has not yet been conclusively demonstrated.

The author wishes especially to thank Dr. Donald D. Van Slyke, Dr. John P. Peters and Dr. Louis Leiter. His thanks are also due Dr. Francis G. Blake, Dr. David M. Kydd, Dr. Walter W. Palmer and Miss Pauline M. Hald.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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Published by  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 154

August 1, 1948

NUMBER 2



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## PLASMA INORGANIC PHOSPHATE CONCENTRATION AND HYPERVENTILATION IN THE DOG<sup>1</sup>

GILBERT S. CAMPBELL, E. B. BROWN, JR., AND FRANK GOLLAN

*From the Department of Physiology, University of Minnesota*

MINNEAPOLIS, MINNESOTA

**H**YPERVENTILATION in man is known to result in a lowering in plasma inorganic phosphate concentration (1, 2, 3) and in urinary phosphate excretion (3, 4). Anesthesia (5, 6) and shock (7) are associated with plasma concentration changes in the reverse direction. In order to be able to study the fate of phosphate retained in the body and lost from the plasma in hyperventilation it seemed important to study the problem in experimental animals. It is technically difficult to hyperventilate unanesthetized dogs mechanically while their respiratory motor mechanisms are functional. This paper is a report of studies on the effects of hyperventilation of dogs under general anesthesia or without anesthesia, employing curare in the latter case to permit overventilation.

### METHODS

Mongrel dogs without special pre-treatment were used. For the experiments under general anesthesia nembutal 30 mgm/kgm. was administered intravenously. Artificial respiration through a tracheal tube was administered by means of a variable speed, variable stroke pump. The arterial blood CO<sub>2</sub> content was measured by the manometric method of Van Slyke and Neill (8). The plasma inorganic phosphate was determined by the method of Fiske and SubbaRow (9). In the experiments with curare 0.6 to 1.0 unit of curare (Intocostin, Squibb) per pound of body weight brought about respiratory paralysis.

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Received for publication June 1, 1948.

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

## RESULTS

Observations on 11 dogs are presented in table 1. In the first two sections of the table the effects of hyperventilation under nembutal and under curare are contrasted. It is apparent that in nembutalized dogs no appreciable change in plasma inorganic phosphate concentration is produced by hyperventilation for periods up

TABLE 1. INFLUENCE OF HYPERVENTILATION ON PLASMA INORGANIC PHOSPHATE IN RELATION TO ANESTHESIA

DOG NO.	ANESTHETIC	CURARE	DURATION OF HYPERVENTILATION	CO <sub>2</sub> CONTENT	INORGANIC PO <sub>4</sub>
			<i>min.</i>	<i>vol. %</i>	<i>mgm. %</i>
1	Nembuta	—	0	53.8	3.3
	"	—	40	37.9	3.1
2	"	—	0	41.1	4.2
	"	—	60	34.2	4.0
3	"	—	0	40.8	3.5
	"	—	120	26.1	3.4
4	"	—	0	46.2	6.9
	"	—	300	27.6	6.6
5	None	+	0	34.8	4.7
	"	+	75	14.6	0.6
6	"	+	0	54.5	3.0
	"	+	90	38.9	0.7
7	"	+	0	35.1	3.0
	"	+	120	24.7	0.6
8	"	+	0	34.1	4.0
	"	+	120	22.0	0.7
9	Local	+	0	43.2	2.8
	"	+	40	40.6	1.7
	"	+	240 <sup>1</sup>	36.0	4.2
	"	+	300	33.6	5.6
10	None	—	0	48.0	4.5
	"	—	(30) <sup>2</sup>	47.4	4.2
11	"	—	0	44.5	4.6
	"	—	(60) <sup>2</sup>	44.4	4.1

<sup>1</sup> Severe hemorrhage in the interval preceeding.

<sup>2</sup> Quiet spontaneous breathing of trained dogs lying supine.

to five hours and of a degree sufficient to reduce the arterial blood CO<sub>2</sub> content by a third. By contrast, without anesthesia the same lowering in arterial CO<sub>2</sub> content by hyperventilation is associated with a decline in plasma inorganic phosphate to a fourth or less of its initial value.

When surgical procedures under local anesthesia are associated with hyperventilation in the curarized dog the changes in plasma inorganic phosphate are not uniform. An example of four such experiments is shown in table 1 in the case of

dog 9. It will be seen that following hemorrhage the plasma inorganic phosphate level rose.

To determine whether simple restraint in the dorsal recumbent posture (10) would produce changes in plasma inorganic phosphate comparable to those seen in hyperventilation, dogs 10 and 11 were so treated. Minimal alterations in the measured plasma constituents occurred.

#### DISCUSSION

The marked decline in plasma inorganic phosphate observed in voluntary (1, 2) or passive (3) hyperventilation in man can be observed also in the dog, if the complications of anesthesia, hemorrhage and shock are obviated. It is important that this point be established because it indicates that the phenomenon is not peculiar to one species, and further because a method is now available for study of the mechanism in experimental animals. This point acquires significance because chronic passive hyperventilation in man (3) results in a net storage of phosphate in the body. The site of that storage may be important in connection with the changes in respiratory function, particularly in sensitivity to the alveolar  $\text{CO}_2$  tension, observed after chronic hyperventilation (11).

#### CONCLUSIONS

Plasma inorganic phosphate concentration was markedly decreased by artificial overventilation of unanesthetized dogs which had received intravenous curare to the point of respiratory paralysis. Hyperventilation of anesthetized animals failed to lower plasma inorganic phosphate. Overventilation of dogs in shock resulted in irregular changes in plasma inorganic phosphate. The restraint of quiet, well trained dogs in the supine position produced only a slight fall in plasma inorganic phosphate.

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# CHRONIC INANITION, RECOVERY, AND METABOLIC RATE OF YOUNG RATS

F. H. QUIMBY<sup>1</sup>, N. E. PHILLIPS AND I. U. WHITE

*From the Department of Zoology, University of Maryland*

COLLEGE PARK, MARYLAND

IT HAS been shown that adult organisms on restricted diets adjust themselves by a reduction in basal metabolism. Klemperer (1), Svenson (2), and Magnus-Levy (3) after clinical observations hypothesized that there is such an adjustment to low caloric diets. Castaldi (4), as a war prisoner, spoke of 'adaptation' to reduced diets. The experimental observations of Pashutin (5) on animals maintained with small rations showed a reduction in respiratory oxygen and carbon dioxide. Morgulis (6) concluded that under the condition of chronic inanition the basal metabolism passes through the same stages of depression recognized in acute inanition. Keys (7) found that men underfed for 24 weeks exhibited a decline of 30 per cent in basal metabolism, or a decline of 10 per cent if calculated per unit weight of active tissue.

Investigations on the metabolic rate in recovery from chronic inanition have been limited to adult animals. Morgulis (6), experimenting with a dog, found that after two weeks of realimentation the metabolic rate increased 100 per cent over that of chronic inanition and 30 per cent above normal. The values did not drop to normal until after five weeks of recovery feeding. Keys *et al.* (8) reported that after 12 weeks of rehabilitation of semi-starved men the basal oxygen consumption was still considerably below the control; after 20 weeks it was slightly higher than the control; and after 32 weeks it was nearly the same as the control. The recovery was closely determined by the caloric intake.

No study has been made of respiratory metabolism during chronic inanition and recovery in young growing animals. It was the purpose of this investigation to determine the effects of chronic underfeeding on the metabolic rate and the respiratory quotient and to follow the course of metabolic recovery during realimentation. Also presented in this report are the alterations in metabolism produced by therapeutic injections of growth hormone, testosterone, and B-complex administered during the recovery period.

## METHODS

Carbon dioxide is an end product of oxidation of all carbonaceous material, aside from those fragments of the protein molecule that are excreted in the urine. A measure of the carbon dioxide production furnishes, therefore, an estimate of total catabolism. Because of the simplicity and consequent accuracy, the metabolic rates in this experiment were calculated from carbon dioxide determinations.

The studies were arranged in two separate experiments. In both experiments young male albino rats, 30 days of age, were employed in groups of 10, each group constituting an experimental

Received for publication May 24, 1948.

<sup>1</sup> Now with the Office of Naval Research, Navy Department, Washington, D. C.

unit. The rats were placed in individual cages and were underfed by restriction of the daily intake of a qualitatively balanced ration to an amount that would just maintain body weight. At the end of the selected periods of underfeeding the animals were put upon full and adequate rations for various periods of time.

Sample metabolic rates were taken of the rats in a state of chronic inanition of 30 days' duration, and after 10, 20, and 30 days of refeeding. Similar determinations were made on other animals after a more prolonged underfeeding of 90 days and at refeeding intervals of two, five, and eight weeks. Determinations were also made on fully fed normal rats. All metabolic data collected were non-fasting; that is, food was not withheld 18 hours before determinations.

A modification of the Haldane open-circuit was employed in making these metabolic studies. Air was drawn through the animal chamber at a rate of 2 l/min. after being made carbon dioxide and water free by passing it through moist soda lime, anhydrous calcium sulfate and activated alumina. Carbon dioxide was collected from the air leaving the animal chamber for a period of two to four hours by the use of Ascarite. Water in the air was collected by the use of a Swartz tube immersed in a bath of dry ice and alcohol. The collecting tubes were weighed on an analytical balance to the nearest milligram, and the cage, with animal, feces and urine, was weighed on a torsion balance to the nearest hundredth gram. Movement of animals was kept at a minimum by the use of bright lights and by permitting the rats to quiet down in the chamber before making the determination. The experiments were conducted during the winter and the determinations were made between 10:00 A.M. and 4:00 P.M. in accordance with Horst *et al.* (9), who showed that oxygen uptake in the rat is abnormally high in the morning and late afternoon, and in accordance with Sherwood (10), who reported that rats exhibit a marked diminution in metabolism during the summer months. The metabolic rates are expressed as the number of calories liberated per square meter of body surface in 24 hours, the latter being calculated as the two-thirds power of the body weight multiplied by 9.1 as a constant. The calories were calculated from an average respiratory quotient based upon preliminary experiments and from the amount of carbon dioxide released.

The administration of growth hormone and testosterone has been described in a previous report by Quimby (11). Vitamin B complex was given daily by intraperitoneal injection in the amount of 0.2 ml. The preparation employed was 'Betalin Complex' (Lilly) and contained in each injection 0.5 mg. thiamin, 0.2 mg. riboflavin, 0.25 mg. pantothenic acid and 0.5 mg. pyridoxine.

RESULTS

TABLE 1. EFFECT OF VARIOUS PERIODS OF REFEEDING AND RECOVERY THERAPY ON THE METABOLIC RATE OF THE RAT<sup>1</sup> (CALORIES PER 24 HOURS PER SQUARE METER BODY SURFACE).  
MEAN AND STANDARD DEVIATION.

EXPER. <sup>2</sup> GROUP	REFED 10 DAYS	REFED 20 DAYS	REFED 30 DAYS
Saline control.....	1710 ±42	1375 ±36	1330 ±54
Growth hormone.....	1395 ±68	1260 ±81	1150 ±49

EXPER. <sup>3</sup> GROUP	REFED 2 WEEKS	REFED 5 WEEKS	REFED 8 WEEKS
Saline control.....	1752 ±32	1517 ±50	1322 ±57
Growth hormone.....	1382 ±12	1414 ±133	1274 ±136
B-complex.....	1710 ±58	1496 ±81	1334 ±70
Testosterone.....	1402 ±78	1466 ±24	1354 ±16

<sup>1</sup> Each group in the tables consisted of 10 animals.

<sup>2</sup> Before refeeding, these rats had an average metabolic rate of 1014 ±96 as a result of a 30-day chronic starvation period.

<sup>3</sup> Before refeeding, these rats had an average metabolic rate of 920 ±102 as a result of a 90-day chronic starvation period. The average metabolic rate of fully-fed normal rats was 1149 ±125.

## DISCUSSION

*Effect of Chronic Inanition on Metabolic Rate.* Table 1 shows that there was about a 10 per cent and 20 per cent reduction in the metabolic rate after 30 days and 90 days of semi-starvation respectively. The caloric restriction employed in this experiment in bringing young rats to a state of chronic inanition was accompanied by a proportional restriction of minerals and vitamins. The amount of thiamin (vitamin B<sub>1</sub>) contained in the daily ration during the underfeeding period was 0.02 milligram. This constituted, according to Griffith and Farris (12), only about one fourth of the minimum daily requirements necessary for normal growth. Although the cause of the depressed metabolism in the underfed rats was not investigated in this experiment, the studies of other workers (13-20) suggest that it may be attributed to the specific deficiency of vitamin B<sub>1</sub>, the effect being mediated through the pituitary and thyroid glands.

*Effect of Chronic Undernutrition on the Respiratory Quotient.* The respiratory quotients of these semi-starved rats were, without exception, low, typically fasting values in determinations made before permitting the daily ration; and they were high, typically non-fasting values in determinations made after consumption of the daily ration. The mean of six fasting determinations was 0.75 and that of six non-fasting determinations was 0.94. Although the type of food restriction in this experiment was purely quantitative, with carbohydrates, fats and proteins available in balanced but inadequate amounts, one might have expected a reduction in the R. Q. on the basis of other studies. For example, Dann and Chambers (21) found an almost complete suppression of the ability to oxidize ingested glucose in dogs after a three-week fast. Chambers (22) noted that as the amount of carbohydrate in the diet was decreased there was a diminished utilization. Marrazzi (23) observed that restriction of food intake decreased absorption of glucose. It is clear, however, that in the young semi-starved rats of this present experiment carbohydrates were being normally if not preferentially absorbed and metabolized as shown by the high respiratory quotients found in determinations made following the ingestion of food. In fact, Quimby (24) has shown that absorption and utilization of food in these animals was actually facilitated by chronic starvation.

*Effect of Refeeding on Metabolic Rate.* The stimulation of metabolism which occurred in early recovery (table 1) must be associated with the intense growth and regeneration of the active protoplasm. The influx of nitrogenous materials which naturally accompanied realimentation might offer an explanation, but this does not appear reasonable in view of the fact that the supply of nourishment continued to be great even as the metabolic rate gradually returned to normal. It is clear therefore that the enhanced metabolism of recovery must be interpreted as due to the active, growing, regenerating tissues. There is no evidence in the literature and no measurements were taken in this experiment which would indicate that the pituitary or thyroid were responsible for this phenomenon.

The metabolic rate was nearly normal at the end of the refeeding periods, although the longer period of undernutrition prolonged the time required for recovery.

*Effect of Growth Hormone and Testosterone on the Metabolic Rate During Refeeding.* The reduction in the metabolic rate during recovery which resulted from growth

hormone injections (table 1) is in agreement with the results obtained on normal animals with similar growth extracts used by Kleiber and Cole (25) and Teel and Cushing (26), whose extracts reduced metabolism while promoting growth. The explanation of this effect, however, must await the evidence of further experimentation.

Injections of testosterone also resulted in a reduction of metabolic rate (table 1). The action in this case was due to the inhibitory effect of the sex hormone on the pituitary gland. In a previous report, Quimby (11) has shown that pituitary depression resulted from the testosterone therapy employed in these animals.

The fact that the testosterone and growth hormone did not maintain their depressing action on the metabolic rate throughout recovery was probably due to the fact that the pituitary recovered in its secretory capacity so as to counteract the inhibitory effects of these substances.

The administration of vitamin B complex was without effect, indicating that amounts adequate for metabolic recovery were present in the food consumed during realimentation.

#### SUMMARY

Chronic starvation in young growing rats lowered the metabolic rate 10 to 20 per cent below normal, but an abnormally high metabolism appeared during the early period of refeeding. The enhanced metabolism of early recovery was decreased in rats treated with growth hormone and with testosterone.

The administration of vitamin B complex did not alter the course of metabolism during recovery. The metabolic rates of all groups were normal or nearly normal at the end of the refeeding periods, although the longer period of undernutrition prolonged the time required for recovery. The non-fasting respiratory quotients of the rats in a state of chronic undernutrition had values slightly higher than those given for normal rats.

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# EFFECTS OF ALTITUDE ANOXIA ON RENAL FUNCTION

ROGER K. McDONALD<sup>1</sup> AND VINCENT C. KELLEY<sup>2</sup>

*From the Department of Medicine, School of Aviation Medicine*

RANDOLPH AIR FORCE BASE, TEXAS

AT THE present time there is a paucity of data available on the effect of reduced atmospheric pressure on the excretory function of the kidney. Alving *et al.* (1) studied the effects on renal function of exposure of young men to simulated altitudes of 10,000 to 18,000 feet for four to six hours daily, six days per week over periods of four to six weeks. They found that such exposure caused no change in renal plasma flow, glomerular filtration rate nor filtration fraction, but did cause invariably an increase in maximum tubular excretory capacity for diodrast ( $Tm_D$ ). In none of eight experiments performed on 5 subjects was a decreased  $Tm_D$  observed. In 2 subjects at 10,000 feet and 11,500 feet, respectively, the increase was within the standard deviation for normal subjects at ground level, but in all other experiments the increases were of greater magnitude. An increased  $Tm_D$  was noted on the thirteenth day of exposure to altitude, the earliest that any subject was studied. In 2 subjects the  $Tm_D$  showed no tendency to return to pre-ascent values for as long as 41 days, the longest time that studies were made at altitude.

The oxygen requirement of the normal kidney has been studied by several investigators. In 1905, Barcroft and Brodie (2, 3) reported the oxygen consumption per minute by single-kidney dogs varied widely but averaged about 1.5 cc., and that diuresis produced by urea and sodium sulfate was accompanied by a large increase of the oxygen absorbed by the kidney. They did not believe that the increased oxygen consumption was attributable to the increased urine output at the glomerulus but stated that, "... it is under these circumstances that the kidney may be most active". Fee and Hemingway (4), using perfused isolated kidney preparations, also reported a rise in renal oxygen consumption with increased urine volume. Hayman and Schmidt (5) reported results of oxygen consumption by the kidney of anesthetized dogs on the cubic centimeters of oxygen per gm. per minute basis similar to those of Barcroft and Brodie, but did not notice any characteristic effect on the apparent oxygen metabolism of the kidney by increased urine volumes produced with caffeine or sodium sulfate. Adolph (6) reported that in frog kidneys a 4 per cent atmosphere of oxygen was sufficient to cause a complete cessation of urine formation. This effect was shown to be due to constriction of the renal arterioles and was not modified by denervation of the kidney. Van Slyke *et al.* (7) were unable to show any relationship of renal blood flow or oxygen consumption to the work of the kidney in excreting urea or water at greatly varying rates. The renal oxygen consumption of the dogs used in their studies varied from 2.0 to 13.3 cc. of  $O_2$  per minute for two-kidney dogs, and 1.1 to 9.5 cc. in one-kidney animals. Thus it is seen that in all *in vivo* studies of renal oxygen consumption reported considerable variations are noted. In regard to the problem of oxygen requirement for excretory work of the kidney Van Slyke *et al.* stated: "It appears that neither the excretory work nor the processes directly connected with it control the oxygen consumption of the kidney, which must be governed by the energy requirements of the non-excretory processes in the organ. This conclusion is explicable by the fact that, as calculated by Brodie, Barcroft and others, the thermodynamic work ordinarily done by the kidney in excretion is less than one per cent of the energy furnished

Received for publication May 28, 1948.

<sup>1</sup> Present Address: U.S.P.H.S. Cardiovascular Diseases and Gerontology Section, Baltimore City Hospitals, Baltimore, Md.

<sup>2</sup> Present Address: Department of Pediatrics, University of Minnesota Medical School.

by the respiration of the organ." These same investigators observed that, in view of the fact that the renal venous blood is normally more than 85 per cent oxygenated, the oxygen tension under which the tissues are maintained is higher than in most other organs.

Toth (8) noted that anoxia in anesthetized dogs induced by the respiration of gas mixtures containing percentages of oxygen below 10 per cent for  $\frac{1}{4}$  to  $2\frac{1}{2}$  hours usually resulted in oliguria, but occasionally in polyuria. By giving infusions of epinephrine to dogs he could produce either oliguria or polyuria depending upon the rate of the infusion. In view of the similar effects of anoxia and epinephrine on the urinary output, Toth proposed that epinephrine was the responsible agent in anoxia for the observed oliguria and polyuria.

Malmejac (9) reported on the effect of anoxia on kidneys explanted to the neck and interposed on the caroticojugular circulation in dogs. He noted that urine secretion began to decrease noticeably at reduced atmospheric pressures equivalent to altitudes between 14,000 and 18,000 feet. At 24,000 feet urine secretion was three quarters of the usual amount. At from 30,000 to 33,000 feet renal blood flow and urine secretion ceased entirely and terminal cardiovascular accidents appeared.

Recent studies (10-14) demonstrate a remarkable resistance of the kidney to prolonged ischemic anoxia with a striking ability of the kidney exposed to recover its normal functions within a short time. Studies based on reduction of renal blood flow are strongly indicative of marked inhibition of the oxidative systems in the kidney under these conditions.

Using the Warburg technique for measuring cellular respiration, it has been demonstrated that the  $QO_2$  (cubic millimeters of oxygen per mg. of dry weight of tissue per hour) of kidney tissue is exceeded only by that of the retina (15-17). This is good indirect evidence that the kidney has a very high oxygen requirement. In studies using the Warburg technique in our laboratories the effect of reduced atmospheric oxygen on the  $QO_2$  of kidney slices was determined (18). In these studies kidney tissue slices 0.3 mm. thick were subjected to atmospheric oxygen concentrations of six per cent in the Warburg apparatus. The  $QO_2$  measured in this situation was two thirds of the  $QO_2$  in air. In this respect the kidney shows no difference from liver and heart muscle which have similar reductions of  $QO_2$  under the same reduced oxygen tensions. These findings are *deduced* to show that although the kidney may have a remarkable ability to maintain its viability under conditions of anoxia, the overall work ability of this organ is not necessarily maintained. Indeed, from the *in vitro* evidence it is conceivable that a lowering of maximum work ability is effected.

The above described studies on the relationship of oxygen requirements of the kidney to the function of this organ may be epitomized as follows. a) Oxygen consumption of the kidney varies widely within the course of an experiment and does not seem to bear a characteristic relationship to excretory work of this organ. b) The oxygen consumption of the kidney is determined primarily, and almost exclusively, by non-excretory processes of the kidney. c) The high degree of oxygen saturation of renal venous blood (85% or more) indicates that renal tissues are normally maintained under higher oxygen tension than most other organs. d) Under conditions of anoxia the urinary output is usually decreased but may be occasionally increased. However, as one approaches a critical level of atmospheric oxygen tension (about 45 mm. Hg in dogs) renal blood flow and urine formation cease and the animal is in a moribund state. e) The normal kidney is remarkably resistant to rather prolonged periods of anoxia. This ability of the kidney to maintain its viability under prolonged periods of anoxia is coupled with the marked inhibition of oxidative systems in the kidney. However, it should be stated that the overall work ability of the kidney under this anoxic condition is not known.

#### EXPERIMENTAL PROCEDURE

Five well trained dogs, divided into two groups, were employed in these studies. All animals were unanesthetized and loosely restrained on a comfortable animal board.

The dogs were fasted for 12 hours before the experiment and were given 50 cc. of water per kg. of body weight one or two hours before the collection periods were begun. An indwelling, mushroom catheter was used in obtaining all urine specimens. Near the end of each collection period the bladder was washed once or twice with 20 or 40 cc. of saline and a comparable volume of air. Manual expression of the bladder was used in conjunction with the saline-air flushings. An additional aid in insuring complete bladder evacuation was the positioning of the animal board on an incline of about  $20^\circ$  with the head elevated. The urine collection periods ranged from 10 to 15 minutes each. Blood was obtained by external jugular puncture and heparin was used as an anticoagulant. The renal function tests were performed at ground level and in an altitude chamber at simulated altitudes of 18,000 feet (79.4 mm. Hg oxygen tension) and 24,000 feet (61.6 mm. Hg oxygen tension).

In *experiment I*, 3 adult, female, mongrel dogs, each weighing about 10 kg., were used. In this experiment creatinine was used to measure the glomerular filtration rate (GF) and para-aminohippuric acid (PAH) was used to measure the effective renal plasma flow ( $C_{PAH}$ ) and the maximum tubular excretory ability ( $Tm_{PAH}$ ). At least 20 minutes before the first period a primer dose of creatinine and PAH was given intravenously and immediately after this an intravenous infusion of creatinine and PAH was begun.

In the creatinine determination a 'correction' based on the initial plasma blank was not used, as the preponderance of data in the literature suggests that endogenous creatinine (creatinine plus other substances giving the Jaffe reaction) is treated quite similarly to exogenous creatinine by the kidney. Any error that might arise from omission of the 'correction' was minimized by maintaining the plasma creatinine levels above 10 mg. per cent in all cases as advocated by Shannon *et al.* (19). Plasma PAH values were maintained between 1 and 3 mg. per cent for measurement of  $C_{PAH}$  and between 30 and 90 mg. per cent for determination of  $Tm_{PAH}$ .

The following procedure was adhered to for the values obtained at ground level (750 feet above sea level) and at simulated altitudes of 18,000 feet and 24,000 feet. Eight urine collections were carried out on each dog, starting five minutes after reaching the simulated altitude. During the first several periods creatinine clearance and PAH clearances were obtained. Each animal was then given a booster dose of PAH intravenously and a new infusion of higher PAH concentration was substituted for the original infusion solution. After 20 to 30 minutes several more urine collection periods were carried out to determine the  $Tm_{PAH}$  values. Thus the elapsed interval of time between the time of reaching the desired altitude and the time of commencing the first of the series of consecutive urine collection periods for determination of  $Tm_{PAH}$  was in all cases approximately 75 minutes. A minimum interval of one week was maintained between successive tests on any one animal, and each dog was tested at only one altitude at any one time.

*Experiment II* was designed to test the changes attributable to altitude by studies in which measurements were made at different altitudes on the same day.

In this group the measurement of the effective renal plasma flow was omitted in order that the experimental procedure might adhere to the following pattern. The  $Tm_{PAH}$  was determined for several consecutive urine collection periods at ground

level pressure in the altitude chamber. Immediately after the last period, the pressure was reduced to a simulated altitude of 18,000 feet. The rate of ascent was standardized at 3000 feet per minute. Five minutes after reaching this altitude the animals were subjected to several more urine collection periods. Immediately after the last period the pressure in the chamber was further reduced to simulate an altitude of 24,000 feet. Here also ascent was at the rate of 3000 feet a minute. Again, several consecutive urine collection periods were carried out five minutes after reaching this altitude.

Two adult, female, Dalmatian coach hounds, each weighing about 22 kg. comprised group 2. In this group inulin was used rather than creatinine to measure the glomerular filtration rate, as we have reason to doubt that creatinine is a completely reliable expression of glomerular filtration in Dalmatian coach hounds (20). The inulin was prepared for intravenous administration in the manner prescribed by Smith *et al.* (21). To insure adequate urine flow for the purpose of avoiding crystallization of the inulin in the urine, the infusion was made to contain sodium sulfate in a concentration of two per cent.

Inulin and para-aminohippuric acid were determined by the method outlined by Smith *et al.* (22). Creatinine was determined by the method of Folin and Wu (23).

#### EXPERIMENTAL RESULTS

*Glomerular filtration.* Table 1 shows the results obtained for the GF at ground level and at altitudes. Moderate variations are noted in the altitude values as compared to the values obtained at ground level, with the exception of dog 3 at 18,000 feet. In this case a rather striking increase in the  $C_{CR}$  was observed. This change, which occurred upon the first exposure of this animal to altitude, was accompanied by overt symptoms of respiratory distress, cyanosis and edema of the paws. Repetition of the  $C_{CR}$  and  $C_{PAH}$  at a later date gave an average  $C_{CR}$  for four consecutive periods of 58.6 and a  $C_{PAH}$  of 164.4.

*Effective renal plasma flow.* The data obtained here (table 1) indicate an increase in the effective renal plasma flow in all dogs at 18,000 feet. At 24,000 feet dog 1 showed a further increase in effective renal plasma flow while dogs 2 and 3 showed a  $C_{PAH}$  decreased below ground level values. From this table it will be seen that no positive correlation is apparent for the changes produced in  $C_{CR}$  and  $C_{PAH}$  at altitude.

*Filtration fraction (FF).* Goldring and Chasis (24) have enumerated various evidences that the filtration fraction is determined primarily by the degree of patency of the efferent glomerular arteriole. If this assumption is correct then certain interpretations of the filtration fraction in the group 1 dogs are justifiable.

The progressive reduction in the FF of dog 1 (table 1) is probably best explained by a decrease in efferent arteriolar tonus (vasodilatation). The possibility of a pyrogenic reaction must be considered as the dog's temperature, although recorded at the start of each experiment, was not followed through the course of the urine collection periods. This would seem somewhat unlikely in view of the fact that the inulin was prepared in the same manner in all cases, the same tubing was used for each dog, and the decreases in the FF occurred on different days.

TABLE I. EFFECTS OF ALTITUDE ON GF, C<sub>PAH</sub>, FF, AND T<sub>MPAH</sub>

DOG NO.	WT.	GROUND LEVEL					18,000 FEET					24,000 FEET				
		Date	GF	C <sub>PAH</sub>	FF	T <sub>MPAH</sub>	Date	GF	C <sub>PAH</sub>	FF	T <sub>MPAH</sub>	Date	GF	C <sub>PAH</sub>	FF	T <sub>MPAH</sub>
1	kg. 8.6	3-11-47	54.5 (5)	161.0 (5)	.343 (5)	9.1 (5)	3-18-47	50.0 (4)	207.1 (4)	.259 (4)	13.1 (4)	4-11-47	67.2 (4)	382.0 (4)	.177 (4)	8.2 (4)
			55.6 (5)					37.5 (4)					68.3 (4)			
			55.0 (10)					43.8 (8)					67.8 (8)			
2	10.5	3-20-47	46.5 (5)	145.0 (5)	.321 (5)	22.6 (4)	3-27-47	44.5 (3)	177.3 (3)	.252 (3)	15.3 (4)	4- 8-47	43.1 (4)	111.5 (4)	.406 (4)	25.8 (4)
			43.0 (4)					49.5 (4)					43.4 (4)			
		9-24-47	44.9 (9)				9-24-47	47.4 (7)					43.3 (8)			
		10-13-47	53.2 (3)			27.0 (7)		58.1 (4)					53.8 (4)			
		10-23-47	39.7 (7)													
			46.1 (7)													
3	8.6	3- 7-47	39.3 (4)	199.2 (4)	.203 (4)	20.1 (4)	3-14-47	104.9 (5)	303.0 (5)	.278 (5)	37.7 (5)	4- 7-47	60.2 (5)	144.3 (5)	.416 (5)	65.1 (4)
			44.2 (4)					92.1 (5)					56.6 (4)			
			41.8 (8)				4-24-47	98.5 (10)	164.4 (4)	.356 (4)			58.6 (9)			
4	21.8	4-21-47	105.9 (5)													
		5- 5-47	97.4 (5)													
		9-15-47	95.5 (4)			87.0 (4)	9-15-47	64.2 (4)			88.9 (4)	9-15-47	63.6 (3)			204.3 (3)
		10-16-46	87.3 (4)													
		11- 3-47	100.8 (4)													
5	22.7	5-19-47	99.8 (10)													
		8- 5-47	110.0 (5)													
		9-18-47	96.0 (3)			85.6 (3)										
		9-23-47	95.0 (4)			75.5 (4)	9-23-47	88.0 (4)			83.2 (4)	9-23-47	61.0 (4)			78.7 (4)
		10-31-47	91.9 (6)													
		11- 6-47	88.0 (3)													

Figures in parentheses refer to the number of periods for which the figure quoted is the mean value.

The values in bold type are averages for the day. It will be noted that on some days there was a considerable difference between the GF values obtained during C<sub>PAH</sub> periods and those obtained during T<sub>MPAH</sub> periods.

In *dog 2* the FF at 18,000 feet was somewhat decreased. The marked rise in the FF at 24,000 feet is strongly indicative of a rather marked vasoconstriction of the efferent glomerular arterioles. The FF of *dog 3* showed a progressively marked increase with an increasing altitude which we interpret as evidence of increasing efferent glomerular arteriolar vasoconstriction.

*Maximum tubular excretory capacity.* The data pertaining to the  $Tm_{PAH}$  values for ground level, 18,000 feet, and 24,000 feet are also shown in table 1. In 4 of the dogs at 18,000 feet and in 3 of them at 24,000 feet no significant changes in  $Tm_{PAH}$  occurred. A suggestive decrease in the  $Tm_{PAH}$  at 18,000 feet as compared to her ground level value was observed in *dog 2*. What significance, if any, should be assigned to this change, viz.,  $Tm$  22.6 to  $Tm$  15.3 is difficult to ascertain in view of the absence of statistical data on normal  $Tm$  variations in dogs. In *dog 3* at 18,000 feet and in *dogs 3* and *4* at 24,000 feet significant increases in the  $Tm_{PAH}$  were observed. These changes were 88 per cent, 222 per cent and 135 per cent, respectively.

#### DISCUSSION

The data presented in this study suggest differences in the effect of altitude on  $C_{CR}$  and  $C_{IN}$  with a tendency to increase in the former and to decrease in the latter. However, these differences must be attributed to individual variation in response since other data on these same animals at altitude fail to corroborate this suggested pattern of response. Indeed, simultaneous observation of  $C_{CR}$  and  $C_{IN}$  at 18,000 feet in one of the mongrel dogs revealed a  $\frac{C_{CR}}{C_{IN}}$  ratio of 1.01.

The effective renal plasma flow was increased in all of the animals upon whom the  $C_{PAH}$  was determined at 18,000 feet. At 24,000 feet the  $C_{PAH}$  was further increased in *dog 1*, while in the remaining 2 dogs it was below the ground level values. This suggests that in an animal exposed to increasing degrees of anoxia a point of maximum effective renal plasma flow is reached and that further increase of anoxia beyond this point results in a lowering of the effective renal plasma flow. The degree of anoxia which will elicit an increased effective renal plasma flow is of course dependent on the individual animal's tolerance and it might be reasonably expected that *dog 1* would show a similar pattern of response at a somewhat greater altitude to that shown by *dogs 2* and *3* at the altitudes studied. In severe conditions of shock and hemorrhage the renal blood flow is diminished by renal vasoconstriction making more blood available for other parts of the body. From this data it appears that a similar situation obtains in the case of severe anoxic stress. The effective renal plasma flow was greatly elevated simultaneously with the remarkably high  $C_{CR}$  in *dog 3* at 18,000 feet, but at 24,000 feet a significant rise in  $C_{CR}$  did not accompany the comparable increase in  $C_{PAH}$  in *dog 1*. Therefore, it may be said that a marked elevation in  $C_{PAH}$  produced by exposure to anoxia does not necessarily imply a concomitantly large rise in the glomerular filtration rate, nor does a decrease in  $C_{PAH}$  entail a corresponding decrease in glomerular filtration rate.

The results obtained by determination of the changes in the filtration fraction in the *group 1* dogs are interesting in that the FF values obtained at altitude differed significantly from the ground level values and in all cases the changes noted were

marked. The fact that no characteristic trend was indicated does not detract from the importance of this observation. Rather it would seem to point up the theory first enunciated by Richards and Plant (25) and later persuasively presented by Toth (8) that the release of epinephrine at varying rates can account for these changes. Although we do not believe that this explanation is necessarily the only acceptable one, we interpret these changes to indicate an alteration of glomerular dynamics by changes in the tonus of the efferent glomerular arterioles.

The whole mechanism of renal circulatory adjustments to conditions of stress seems to be set to insure an effective glomerular filtration rate. If this is the case, the changing FF is a convenient numerical index of the progress of these adjustments. The effective renal plasma flow and the glomerular filtration rate are indices of the effectiveness of this mechanism; the former of its service to the circulation as a whole, the latter of its effectiveness in maintaining kidney function.

The decrease of the available oxygen to the kidney tubules produced in these studies does not result in a noticeably decreased maximum tubular excretory ability. Indeed, the increases in  $Tm_{PAH}$  observed in 3 of the 5 dogs point to an apparent increase in the ability of the tubule cells to excrete PAH upon exposure of the animal to an atmosphere of sufficiently reduced oxygen tension. These observations agree with those of Alving *et al.* (1) in the case of chronic, intermittent exposure to anoxia.

An increase in the maximum tubular excretory ability has been effected by the administration of anterior pituitary lobe extract and thyroid hormone to normal and hypophysectomized dogs (26). Eiler *et al.* (27) produced tremendous increases in  $Tm_D$  by administration of thyroxine. Using testosterone propionate Welsh *et al.* have been able to increase the  $Tm_D$  in dogs up to 100 per cent (28). These studies indicate that hormonal influences are capable of causing alterations in the tubular transfer mechanism of the kidney. The possibility of a hormonal factor being instrumental in causing the elevation of the  $Tm_{PAH}$  in the dogs used in this study must be considered.

The two mechanisms by which an increase in  $Tm_{PAH}$  could be effected are *a*) an increase in the number of participating nephrons and *b*) an actual increase in the ability of the tubular cells to transfer PAH. If the former were the case, the ratio  $\frac{GF}{Tm_{PAH}}$  would remain unchanged; if the latter were the case, this ratio would be reduced. It will be noted from table 1 that in *dog 3* at 18,000 feet both the  $C_{IN}$  and  $Tm_{PAH}$  are increased to approximately twice the ground level values and therefore the ratio  $\frac{GF}{Tm_{PAH}}$  remains roughly constant. However, in the other instances in which an elevated  $Tm_{PAH}$  was observed no corresponding increase in GF occurred, and therefore this ratio decreased markedly. Thus it can be stated that in two of the three experiments in which an increased  $Tm_{PAH}$  was observed this increase is attributable to an increased ability of the tubular cells to transfer PAH, whereas in the third case an increase in the number of participating nephrons is implicated. In this regard, it is interesting that Alving *et al.* (2) found their increases in  $Tm_D$  in the absence of changes in GF, thereby implicating an increase in the ability of the tubular cells to secrete rather than an increase in the number of participating nephrons.



## SUMMARY

Five dogs were subjected to renal function studies at ground level and at simulated altitudes of 18,000 feet and of 24,000 feet. In all cases  $C_{PAH}$  determinations were started five minutes after reaching the desired altitude. In the case of the group 2 animals  $Tm_{PAH}$  measurements were likewise started five minutes after attaining the desired altitude, while in the case of the group 1 animals the  $Tm_{PAH}$  measurements were started approximately 75 minutes after attaining the desired altitude. The glomerular filtration rate in these animals was either increased, decreased or unaffected depending upon the reaction of the individual animal to reduced ambient pressure. The effective renal plasma flow was increased in all dogs at an altitude of 18,000 feet and was further increased in one dog but decreased below the ground level values in the remaining dogs at 24,000 feet. The maximum tubular excretory ability was markedly increased at 18,000 feet in 1 animal and at 24,000 feet in 2 of the 5 animals studied.

The authors are grateful to Dr. Eric Ogden for his assistance and advice during the course of this investigation.

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# FURTHER OBSERVATIONS ON THE EFFECTS OF ALTITUDE ANOXIA ON RENAL FUNCTION

VINCENT C. KELLEY<sup>1</sup> AND ROGER K. McDONALD<sup>2</sup>

*From the Department of Medicine, School of Aviation Medicine*

RANDOLPH AIR FORCE BASE, TEXAS

IN A previous communication (1) we have reported the results of investigations conducted in our laboratory on the effects of altitude anoxia on clearances of inulin, creatinine, and para-aminohippuric acid and on the maximum tubular excretory capacity for para-aminohippuric acid. In these investigations the glomerular filtration rate was either increased, decreased or unaffected depending upon the reaction of the individual animal to exposure to reduced barometric pressure; the effective renal plasma flow as determined by  $C_{PAH}$  was increased at an altitude of 18,000 feet in all dogs and was further increased in one dog but decreased below the ground level values in the remaining 2 dogs at 24,000 feet. The maximum tubular excretory ability was markedly increased in one dog at 18,000 feet and at 24,000 feet in 2 of the 5 animals studied.

The present studies constitute an extension of these investigations to include the effects of altitude anoxia on the maximum rate of tubular reabsorption of glucose ( $Tm_G$ ) and on the simultaneous  $Tm_G$  and  $Tm_{PAH}$ .

The concept of a maximum rate of tubular reabsorption of glucose was established by Shannon and Fisher (2) who demonstrated that at high levels of plasma glucose a definite and constant amount of glucose in mg/min. is reabsorbed by the tubules irrespective of variations of the plasma glucose levels above the minimum level required to saturate the reabsorptive mechanism.

Shannon (3) states that "relatively few precautions need be followed for valid measurements of glucose  $Tm$  provided the animal is well hydrated and the arterial plasma glucose is maintained at an adequate and fairly constant value. Under these conditions the system in the dog has surprising stability, and the glucose  $Tm$  of any animal is quite constant over a period of many months. Excessive insulin may acutely depress it, but it is not affected by epinephrine nor by marked changes in dietary regime, and it is not related to the concurrent rate of glomerular filtration." It has been recently demonstrated that the administration of thyroxine greatly increases the  $Tm_G$  (4).

Klopp, Young, and Taylor (5) have found that high plasma levels of glucose may decrease the  $Tm_{PAH}$ , that high plasma levels of PAH may increase the  $Tm_G$ , and that high plasma levels of both simultaneously may increase the glomerular filtration rate. Houck (6) has reported that  $Tm_G$  and  $Tm_{PAH}$  are both depressed when measured simultaneously with  $Tm_{PAH}$  depressed approximately twice as much as  $Tm_G$ . The magnitude of the changes observed was not great in either case in this study.

## EXPERIMENTAL PROCEDURE

Three well trained, adult female dogs, one mongrel and 2 Dalmatian coach hounds, were used in these studies. The dogs were unanesthetized and loosely re-

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Received for publication May 28, 1948.

<sup>1</sup> Present Address: Department of Pediatrics, University of Minnesota Medical School.

<sup>2</sup> Present Address: U.S.P.H.S. Cardiovascular Diseases and Gerontology Section, Baltimore City Hospitals, Baltimore, Md.

strained on a comfortable animal board. The technique used in obtaining blood and urine specimens and insuring complete evacuation of the bladder was the same as previously outlined (1). The renal function tests were performed in an altitude chamber at ground level and at simulated altitudes of 18,000, 24,000, and 28,000 feet (one test).

The procedure followed in each case in studying the effect of altitude anoxia on  $Tm_G$  was as follows: after the dog was placed in the altitude chamber on the animal board, a primer dose of glucose and inulin was given intravenously and immediately a constant infusion of glucose and inulin was started. (In the case of the mongrel, *dog 3*, creatinine was used rather than inulin.) At least 20 minutes later the first urine collection period was begun. Several periods were run at ground level. The pressure was then decreased to a simulated altitude of 18,000 feet at a rate of ascent of 3000 feet per minute, and after approximately 10 minutes the first period at this altitude was commenced. Upon the completion of several periods further ascent to a simulated altitude of 24,000 feet was accomplished at the same rate of ascent and after an interval of 10 minutes several more periods were carried out at this altitude.

In the studies of simultaneous  $Tm_G$  and  $Tm_{PAH}$  the primer and infusion solutions contained sodium para-aminohippurate in addition to the materials mentioned above. The procedure in these studies was the same as above with the exception that no studies were made at 18,000 feet, but following the periods at ground level the dogs were taken directly to an altitude of 24,000 feet at the rate of 3000 ft/min. In one case (*dog 2*) at the completion of four periods at 24,000 feet the dog was taken to an altitude of 28,000 feet and three more periods were carried out.

Inulin and para-aminohippuric acid were determined by the methods outlined by Smith (7). Creatinine was determined by the method of Folin and Wu (8). Glucose was determined by the Folin method (9). All measurements were made on an Evelyn photoelectric colorimeter.

#### EXPERIMENTAL RESULTS

The data obtained regarding the effect of exposure of the animals to altitude anoxia on their maximum tubular reabsorptive ability are shown in table 1. It will be noted that at 18,000 feet there was in one animal a relatively small decrease in  $Tm_G$  of approximately 20 per cent, in one animal a larger decrease in  $Tm_G$  of approximately 55 per cent, and in one animal no change in  $Tm_G$  in two separate sets of experiments. It is interesting to note that although there were quite definite changes in  $Tm_G$  at 18,000 feet as compared to the ground level values, there were no appreciable differences observed in any of the animals between the values obtained at 18,000 feet and those obtained at 24,000 feet. It is also interesting that in no case was there any marked change in the glomerular filtration rate at altitude as compared to the ground level values.

The data obtained in the studies of simultaneously determined  $Tm_G$  and  $Tm_{PAH}$  are shown in table 2. In those studies conducted under conditions of normal atmospheric pressure (ground level) a distinct depression of the  $Tm_{PAH}$ , as compared to the  $Tm_{PAH}$  of the animals when determined in the absence of high glucose plasma levels, was observed in all animals. These observations are in agreement with the previous reports of Klopp *et al.* (5) and of Houck (6). Repetition of these experiments at

24,000 feet produced no significant changes in the values of  $Tm_{PAH}$  found. In other words, when  $Tm_{PAH}$  and  $Tm_G$  were simultaneously determined there resulted a marked depression of the dog's normal  $Tm_{PAH}$  whether or not the animal was being exposed to reduced barometric pressure; the extent to which the  $Tm_{PAH}$  was depressed did not seem to be related to the percentage of atmospheric oxygen to which the animal was being subjected.

The values of  $Tm_G$  obtained when  $Tm_G$  and  $Tm_{PAH}$  were simultaneously determined at ground level were in two cases (*dogs 2 and 3*) in good agreement with the values obtained when the  $Tm_G$  was determined in the absence of high plasma levels

TABLE 1. EFFECT OF ALTITUDE ON  $Tm_G$ 

DOG NO.	WT.	DATE	GROUND LEVEL		18,000 FEET		24,000 FEET	
			GF	$Tm_G$	GF	$Tm_G$	GF	$Tm_G$
	kg.							
1	22.7	10-8-47	85.2 (3)	394.3 (3)	86.0 (3)	316.0 (3)	92.5 (4)	319.6 (4)
2	21.8	9-29-47	94.9 (4)	352.3 (4)	88.5 (4)	164.7 (4)	106.2 (4)	155.9 (4)
3	10.5	10-1-47	42.8 (4)	157.9 (4)	44.3 (4)	126.6 (4)	46.4 (4)	143.7 (4)
		10-9-47	48.1 (4)	134.9 (4)	42.8 (4)	137.5 (4)	47.2 (4)	126.5 (4)

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

TABLE 2. EFFECT OF ALTITUDE ON SIMULTANEOUSLY DETERMINED  $Tm_G$  AND  $Tm_{PAH}$ 

DOG NO.	WT.	DATE	GROUND LEVEL			24,000 FEET			28,000 FEET		
			GF	$Tm_G$	$Tm_{PAH}$	GF	$Tm_G$	$Tm_{PAH}$	GF	$Tm_G$	$Tm_{PAH}$
	kg.										
1	22.7	10-14-47	107.1 (4)	236.7 (4)	10.9 (4)	108.3 (4)	205.9 (4)	7.3 (4)			
		10-22-47	61.9 (4)	187.9 (4)	25.1 (4)	67.2 (4)	168.0 (4)	30.0 (4)			
2	21.8	10-10-47	59.9 (6)	333.5 (6)	37.8 (6)	106.1 (4)	256.8 (4)	21.2 (4)	83.9 (3)	182.6 (3)	24.5 (3)
3	10.5	10-29-47	39.8 (4)	151.2 (4)	0.3 (4)	42.5 (4)	147.7 (4)	-2.3 (4) <sup>1</sup>			

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

<sup>1</sup> Range for 4 periods was -0.6 to -5.2. Negative values for  $Tm_{PAH}$  have recently been reported (11). We have also noted an occasional negative  $Tm_{PAH}$  in other experiments.

of PAH; however, in the case of *dog 1*, in two separate sets of experiments at ground level, a rather marked decrease in  $Tm_G$  (40 per cent on one occasion and 52 per cent on one occasion) as compared to the values obtained in the absence of high plasma levels of PAH was noted. This observation is in disagreement with the previous report of Klopp *et al.* (5) that  $Tm_G$  is either unaffected or increased by the presence of high plasma PAH levels but in agreement with the findings of Houck (6).

Comparison of the  $Tm_G$  values obtained at ground level and at 24,000 feet reveals that in the presence of high plasma levels of PAH the tendency toward decreasing  $Tm_G$  with increasing altitude is still apparent, as was the case in the absence of high plasma levels of PAH, but this tendency is considerably less marked. For example, in the case of *dog 2* in the absence of high plasma levels of PAH the  $Tm_G$  was reduced to 47

per cent of the ground level value at 18,000 feet and to 44 per cent of the ground level value at 24,000 feet, whereas in the presence of high plasma levels of PAH it was reduced only to 77 per cent of the ground level value at 24,000 feet and even at 28,000 feet only to 55 per cent of the ground level value.

Table 3 summarizes the data we have obtained showing the effect of altitude anoxia on  $Tm_G$  both in the presence and in the absence of high plasma levels of PAH and its effect on  $Tm_{PAH}$  both in the presence and in the absence of high plasma levels of glucose. The data shown in this table on the effect of altitude on  $Tm_{PAH}$  in the

TABLE 3.  $Tm_G$  AND  $Tm_{PAH}$  DETERMINED INDIVIDUALLY AND SIMULTANEOUSLY AT VARIOUS ALTITUDES

DOG NO.	WT.	DATE	DETERMINATION	GROUND LEVEL		18,000 FT.	24,000 FT.	
				Individually	Simultaneously	Individually	Individually	Simultaneously
1	kg. 22.7	10-8-47	$Tm_G$	394.3 (3)		316.0 (3)	319.6 (4)	
		10-14-47	$Tm_G$		236.7 (4)			205.9 (4)
		10-22-47	$Tm_G$		187.9 (4)			168.0 (4)
		9-18-47	$Tm_{PAH}$	85.6 (3)				
		9-23-47	$Tm_{PAH}$	75.5 (4)		83.2 (4)	78.7 (4)	
		10-14-47	$Tm_{PAH}$		10.9 (4)			7.3 (4)
		10-22-47	$Tm_{PAH}$		25.1 (4)			30.0 (4)
2	21.8	9-29-47	$Tm_G$	352.3 (4)		164.7 (4)	155.9 (4)	
		10-10-47	$Tm_G$		333.5 (6)			256.8 (4)
		9-15-47	$Tm_{PAH}$	87.0 (4)		88.9 (4)	204.3 (3)	
		10-10-47	$Tm_{PAH}$		37.8 (6)			21.2 (4)
3	10.5	10-1-47	$Tm_G$	157.9 (4)		126.6 (4)	143.7 (4)	
		10-9-47	$Tm_G$	134.9 (4)		137.5 (4)	126.5 (4)	
		10-29-47	$Tm_G$		151.2 (4)			147.7 (4)
		3-20-47	$Tm_{PAH}$	22.6 (4)				
		3-27-47	$Tm_{PAH}$			15.3 (4)		
		4-8-47	$Tm_{PAH}$				25.8 (4)	
		10-13-47	$Tm_{PAH}$	27.0 (7)				
		10-29-47	$Tm_{PAH}$		0.3 (4)			-2.3 (4)

Figures in parentheses indicate the number of periods for which the figure quoted is the mean value.

absence of high plasma levels of glucose are taken from a previous publication (1) and are introduced at this point merely for purposes of comparison. It will be noted from this table, in addition to the points previously stressed, that in *dog 2* the immense increase of  $Tm_{PAH}$  at 24,000 feet as compared to the ground level value reported in our previous paper is not apparent in the present studies where  $Tm_G$  and  $Tm_{PAH}$  are simultaneously determined. It is not possible to state whether the failure of this interesting finding to recur is attributable to the high plasma levels of glucose in the present experiments. We feel that it could conceivably be ascribed to the high plasma levels of glucose, not on the basis of competition between PAH and glucose with regard to the tubular transfer mechanism but merely on the basis of the improved altitude

tolerance commonly known to be mediated by the administration of glucose. We have tried on numerous occasions to duplicate results of one day's experiment at altitude on another day, both with regard to renal function studies and with regard to other investigations in which we have been interested, and have found this to be a virtual impossibility if the same criteria of satisfactory reproduction of results are employed as those invoked at ground level. The variations of results at altitude from day to day are considerably greater than those observed under conditions of normal atmospheric pressure although, in general, the results are qualitatively and within a certain degree of accuracy quantitatively reproducible. This fact may be attributed to any or all of several factors (10), but it must be borne in mind that a given animal's tolerance to anoxic exposure varies from day to day. It has seemed to us that the most pronounced changes in renal function have been observed on the days when the dogs have appeared to be the most distressed by exposure to altitude. Therefore, it seems possible that the high plasma glucose level may have been important in preventing the occurrence of elevation of the  $Tm_{PAH}$  value at altitude during the experiment in which  $Tm_{PAH}$  and  $Tm_G$  were simultaneously determined. However, one cannot be certain that this phenomenon would have recurred even in the absence of a high plasma level of glucose.

Examination of the values obtained for glomerular filtration rates in the experiments in which  $Tm_G$  and  $Tm_{PAH}$  were simultaneously determined reveals some interesting changes that occurred in this regard. In the case of *dog 1* on one experimental day a GF of 107 was observed and on another day a GF of 62 was found. In neither case was this value appreciably altered upon exposure to altitude. This dog's normal GF has been established by numerous determinations (34 periods) to average 98.1 with a range of from 88 to 110. In the case of *dog 2* a GF of 60 was observed at ground level. This value was increased to 106 at 24,000 feet and decreased to 84 at 28,000 feet. This dog's normal GF has been established (22 periods) to average 97.7 with a range of 87.3 to 105.9. Thus in these two cases the  $C_{IN}$  was definitely decreased below the normal values for these dogs. In this respect we again find our results at variance with those of Klopp *et al.* who found an increase of glomerular filtration rate over the normal value during their experiments in which  $Tm_G$  and  $Tm_{PAH}$  were simultaneously determined.

#### SUMMARY

Three dogs were submitted to renal function tests at ground level and at simulated altitudes in an altitude chamber. The tests performed were determination of  $Tm_G$  alone and simultaneous determination of  $Tm_G$  and  $Tm_{PAH}$ . In 2 of the 3 animals a decrease in  $Tm_G$  was observed at 18,000 feet as compared to the ground level values, but in the 3rd animal no change was evident. In none of the animals was there any appreciable difference between the value obtained at 18,000 feet and the value obtained at 24,000 feet.

When  $Tm_{PAH}$  and  $Tm_G$  were simultaneously determined there resulted a marked depression of the dog's normal  $Tm_{PAH}$  whether or not the animal was being exposed to reduced barometric pressure, and the extent to which the  $Tm_{PAH}$  was depressed bore no evident relationship to the altitude to which the animal was being subjected.

In one animal a marked depression of the  $Tm_G$  resulted when  $Tm_G$  and  $Tm_{PAH}$  were simultaneously determined at ground level, but in the other 2 animals no such depression was noted. At high plasma levels of PAH the tendency toward decreasing  $Tm_G$  with increasing altitude, as in the case of  $Tm_G$  determinations in the absence of high plasma levels of PAH, was still apparent but less pronounced. In 2 of the 3 dogs at ground level, the glomerular filtration rate was distinctly depressed in the presence of high plasma levels of PAH and of glucose simultaneously. In one of these dogs exposure of the animal to altitude caused the GF to return to normal, but in the other case it did not do so.

The authors are grateful to Dr. Eric Ogden for his assistance and advice during the course of this investigation.

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# EFFECTS OF ALTERATIONS IN BODY TEMPERATURE ON PROPERTIES OF CONVULSIVE SEIZURES IN RATS<sup>1,2</sup>

EWART A. SWINYARD AND JAMES E. P. TOMAN

*From the Departments of Pharmacology and Physiology, University of Utah College of Medicine and College of Pharmacy*

SALT LAKE CITY, UTAH

IT IS well known that convulsive seizures may frequently be associated with acute febrile illnesses in early childhood, but less frequently in adult life (1, 2). Convulsions have also been reported to occur in patients subjected to fever therapy (3-5). In some epileptic subjects the frequency of seizures may be increased during fever, while in others the attack rate may be diminished (2). Experimentally, acute hyperpyrexia has been shown by Wegman (6) to cause convulsions in kittens. With regard to low body temperature, human refrigeration has been reported by Fay (7) to increase the excitability of the deep reflexes. Similarly, Barron and Matthews (8), and Ozorio De Almeida (9, 10) have shown that a reduction in body temperature of frogs increases the excitability of the spinal cord. In peripheral nerve Granit and Skoglund (11) have observed facilitation of ephaptic transmission, and Lorente de Nó (12) has shown that the rheobase is decreased when temperature is reduced.

Because of the paradoxical occurrence of febrile convulsions in some patients in contrast to febrile remission of seizures in others, it seemed important to analyze the effect of alterations in body temperature on various properties of experimental seizures without the complications, such as infection, dehydration, etc., which may attend febrile illness.

## METHODS

Body temperature of Sprague-Dawley rats was altered by restraining the animals in circular wire mesh holders either in a refrigerator at a temperature of  $-8^{\circ}\text{C}$ . or in an insulated heating cabinet at  $55^{\circ}\text{C}$ . until the desired rectal temperature was obtained. Rectal temperatures were determined with a mercury thermometer immediately before and after experimental seizures.

Electroshock seizures were produced by a 60-cycle alternating current apparatus designed by Dr. Lowell A. Woodbury; the current delivered is independent of the external resistance. Shocks were of 0.2 second duration, and were delivered through corneal electrodes. Minimal electroshock seizure thresholds (13) were compared at control and experimental body temperatures, with a period of at least 12 hours between tests for each animal. For observation of changes in pattern and duration of

Received for publication July 15, 1948.

<sup>1</sup> This investigation was supported from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

<sup>2</sup> A preliminary report appeared in *Federation Proc.* 7: 258, 1948.



maximal seizures (14), supramaximal shocks of 150 mA. (or five times threshold) were given; the interval between tests was at least two hours.

To determine the effect of altered temperature on rate of recovery of maximal seizure pattern, groups of 4 to 12 rats were used at each desired body temperature. All were given a conditioning supramaximal shock, followed by a supramaximal test shock after the desired interval. The percentage of animals showing full recovery of seizure pattern, including the tonic extensor component, was noted. Percentage recoveries for various intervals were plotted on probit paper as a function of time, and

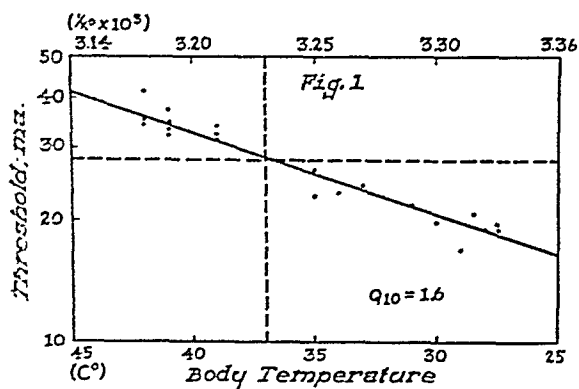


Fig. 1. EFFECT OF BODY TEMPERATURE on the threshold for minimal electroshock seizures in rats. Electroshock threshold in mA. is plotted on a logarithmic scale (ordinate) as a function of the reciprocal of the absolute temperature (*abscissa*, top). For ease in interpretation the corresponding centigrade degrees are also shown (*abscissa*, bottom). Vertical broken line: normal body temperature (average of 21 rats); horizontal broken line: normal electroshock threshold (average of 159 rats). Each point along solid diagonal line represents one experiment.

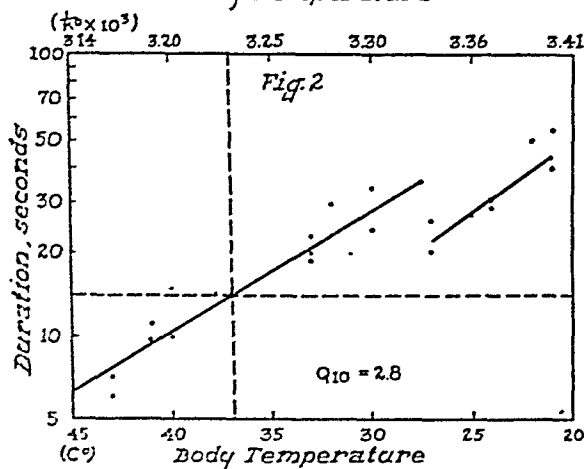


Fig. 2. EFFECT OF BODY TEMPERATURE on the duration of maximal electroshock seizures in rats. Electroshock threshold in mA. is plotted on a logarithmic scale (ordinate) as a function of the reciprocal of the absolute temperature (*abscissa*, top). For ease in interpretation the corresponding centigrade degrees are also shown (*abscissa*, bottom). Vertical broken line: normal body temperature (average of 22 rats); horizontal broken line: total duration of maximal seizures at normal body temperature (average of 73 rats). Each point along solid diagonal line represents one experiment.

the resulting points fitted by eye to determine graphically the time for recovery of full seizure pattern in 50 per cent of the animals at each body temperature.

For determination of the rate of recovery of minimal seizure threshold, the method was the same except that an arbitrary value of 150 per cent of the unconditioned threshold at the same temperature was selected for the test shock.

Chemoshock seizure thresholds for intraperitoneally injected Metrazol or picrotoxin were determined by treating groups of four or more animals at each of several dose levels at each desired temperature and finding graphically (as above) that quantity of drug which would convulse 50 per cent of the animals at each temperature.

## RESULTS

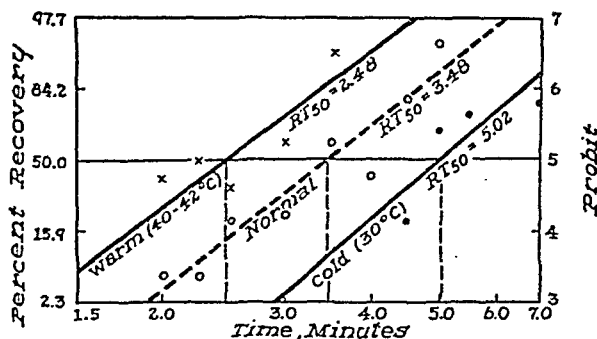
The minimal electroshock seizure threshold was found to vary directly with body temperature. When the logarithm of threshold was plotted against the reciprocal

of the absolute temperature (fig. 1.), the data were approximately fitted by a straight line giving a  $\mu$  value of 7500 calories per mole, corresponding to a  $Q_{10}$  of 1.6.

The susceptibility to convulsions induced by picrotoxin or Metrazol was also increased by lowering the body temperature. The approximate  $Q_{10}$  for picrotoxin was found to be 1.6, which is in agreement with the electroshock data. Occasional spontaneous seizures were observed when body temperature was elevated above 43°C. or reduced below 27°C. This would tend to compromise any observations taken beyond these limits.

The effect of body temperature on the total duration of maximal seizures is shown in figure 2. As might be expected the total duration varies inversely with the body temperature. The  $Q_{10}$  was found to be 2.8 ( $\mu$  value, 19,600 calories per mole). It is interesting to note that a sharp reduction in total seizure duration occurs when body temperature is reduced below 27°C., as shown by the separate line in the upper right segment of figure 2. It should be mentioned that spontaneous convulsions were occasionally observed at and below this temperature.

Fig. 3. EFFECT OF BODY TEMPERATURE on time for recovery of maximal electroshock seizure pattern in rats. Each point (x, open circle or solid circle) on the graph represents a group of 4 to 12 rats. The percentage recoveries (left ordinate; corresponding probit values, right ordinate) are plotted as a function of time (abscissa) and the points fitted by eye.



Associated with the effect of variations in temperature on total seizure duration, changes in the relative duration of the various seizure components were observed. In general, a reduction in temperature decreased the fractional duration of the tonic phase and particularly the initial flexor component of the tonic phase. At elevated body temperatures the absolute as well as the relative duration of tonic flexion was increased; above 42°C. the entire seizure tended to be a tonic flexion with superimposed fine clonus. It was difficult to measure the duration of the various components at temperatures below 27°C. because the end-points were not clearly defined.

The effect of body temperature on the time for recovery of a full maximal seizure pattern following a supramaximal shock is shown in figure 3. It may be seen that the recovery time for 50 per cent of normal rats is  $3.48 \pm 0.25$  minutes. When the body temperature was elevated to 40° to 42°C. the recovery time for 50 per cent of the animals was found to be  $2.48 \pm 0.25$  minutes. Conversely when body temperature was reduced to 30°C. it required  $5.02 \pm 0.44$  minutes for 50 per cent of the animals to recover the maximal seizure pattern. Therefore the  $Q_{10}$  between 30°C. and 40°C. is approximately 2 ( $\mu$  value of 12,000 calories per mole) for recovery of the normal seizure pattern.

Following a maximal seizure, the rate of recovery of threshold was found to be doubled by a 10° reduction in body temperature, giving a  $Q_{10}$  of 2. This is in agreement with the results of the maximal shock experiments.

## DISCUSSION

It is of interest to note that the temperature coefficients differ for seizure threshold, seizure duration and post-seizure recovery. This would seem to indicate a difference in the fundamental chemical processes underlying these three functions (15), but the identification of the specific temperature coefficients with particular limiting enzyme steps would be unwarranted (16).

Since seizure threshold was increased by a rise in body temperature, this factor alone might conceivably account for febrile remission of convulsive disorders in some patients. It obviously could not account for febrile onset of seizures in other cases. Spontaneous seizures were occasionally seen at high body temperatures in the present study, demonstrating that threshold is not the only factor determining the occurrence of convulsions.

## SUMMARY

Characteristics of experimental seizures were studied in rats whose body temperatures were altered by exposure to extreme environmental temperatures. Seizure threshold was increased, seizure duration reduced and post-seizure recovery hastened by increased body temperature, and conversely changed by decreased body temperature. The data were adequately fitted by plotting the logarithm of each function against the reciprocal of absolute body temperature. For seizure threshold the  $Q_{10}$  was found to be 1.6, for seizure duration 2.8 and for recovery 2.0. The possible significance of the findings is briefly discussed.

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# EXCRETION OF BILIRUBIN AND BROMSULFALEIN IN BILE

A. CANTAROW, C. W. WIRTS, W. J. SNAPE AND L. L. MILLER<sup>1</sup>

*From the Departments of Biochemistry, Physiology and Medicine, Jefferson Medical College*

PHILADELPHIA, PENNSYLVANIA

**I**N CONTRAST to the abundance of information regarding the rate of disappearance from the blood of intravenously injected bilirubin and bromsulfalein, relatively little is known concerning the rate of their excretion in the bile. Practically all of the few reported data bearing on this problem have been obtained under conditions that are not entirely satisfactory for one or more of the following reasons: *a)* the effect of anesthesia and reaction to operation in acute experiments; *b)* the inability to maintain consistently normal nutrition and liver function in the usual external bile-fistula dog; *c)* the use of inaccurate methods for determination of pigment in bile.

The most important difficulty has been overcome by the use of the Thomas-type tubulated duodenal-fistula dog (1-3), in which normal nutrition and liver function can be maintained for periods of many months to several years. Moreover, accurate methods are now available for determination of total pigment and bromsulfalein in bile. The present study was undertaken for the purpose of securing information regarding the normal rate of excretion of pigment in the bile and the rate of excretion of pigment and bromsulfalein after intravenous injection of bilirubin and bromsulfalein, singly and in combination. These experiments are to serve as controls for a subsequent study of the influence of various choleretic agents upon the biliary excretion of these substances.

## *Materials and Methods*

Ten trained, cholecystectomized dogs were used, provided with gastric and duodenal fistulae fitted with large cannulae, as described by Thomas (1). The duodenal fistula was placed opposite the ampulla of Vater and bile was collected by inserting a temporary glass cannula (2, 3) into the common duct. The bile was allowed to drain into graduated tubes until the flow became constant. It was then collected in 15-minute samples.

In control studies, collections were continued for periods ranging from 2 to 4 hours. In experiments involving intravenous injection of bilirubin or bromsulfalein, bile was collected for 30 to 60 minutes before injection and for varying periods up to 6 hours subsequently. In experiments involving continuous injection, these were made with a motor-driven constant infusion apparatus, at measured rates, after injecting a priming dose. Bilirubin (Eastman Kodak Co.) was injected in one per cent  $\text{Na}_2\text{CO}_3$  solution (0.3-2.0 mg/cc.). In continuous injection experiments, the commercial bromsulfalein solution was diluted with 0.85 *N* NaCl solution. The amounts injected are indicated in the tables.

All determinations of pigment and dye were made with the Evelyn photoelectric colorimeter. Each 15-minute bile sample was made up to 5 or 10 cc. with distilled water. Total bile pigment was determined by the method of Malloy (4), bromsulfalein in bile, by a method described by Cantarow

Received for publication June 28, 1948.

<sup>1</sup> Present address: School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

and Wirts (5); bilirubin in serum, by the method of Malloy and Evelyn (6); and bromsulfalein in blood, by the method of Bradley *et al.* (7).

### RESULTS AND COMMENT

**Controls.** Data regarding bile volume and pigment output in 8 untreated dogs during 44 periods of at least one hour are presented in table 1. The bile flow ranged from 2.1 to 17.6 cc/hr., 0.140 to 1.257 cc/kg/hr. The bile pigment concentration ranged from 12 to 161 mg/100 cc., and the output from 1.0 to 5.943 mg/hr., 0.019 to 0.289 mg/kg/hr. Both volume and pigment varied considerably in individual animals. For example, the findings in *dog 6*, studied on 16 different days, were as follows: bile volume, 2.1 to 15.2 cc/hr. (0.105–0.760 cc/kg/hr.); pigment concentration, 23 to 153 mg/100 cc.; pigment output, 1.870 to 4.281 mg/hr. (0.093–0.214 mg/kg/hr.).

Early in this study it was found that the presence of food in the stomach was accompanied by wide fluctuations in bile output, pigment concentration and, occasionally, total pigment output in 15-minute periods. This was attributed to the

TABLE 1. BILE VOLUME AND PIGMENT EXCRETION IN UNTREATED DOGS

DOG	NO. OF DETERMINATIONS	VOLUME		PIGMENT		
		cc/hr.	cc/kg/hr.	mg. %	mg/hr.	mg/kg/hr.
3	1	6.0	0.283	60	3.63	0.172
4	6	3.1–13.7	0.206–0.913	12–92	1.372–3.220	0.091–0.214
5	4	3.7–8.1	0.205–0.450	32–77	2.614–4.810	0.145–0.267
6	16	2.1–15.2	0.105–0.760	23–153	1.870–4.281	0.093–0.214
7	1	7.4	0.616	47	3.474	0.289
8	3	4.6–6.5	0.383–0.541	21–57	1.333–2.600	0.111–0.216
9	12	3.3–17.6	0.235–1.257	17–154	1.000–5.943	0.071–0.218
10	1	2.1	0.140	161	3.38	0.225

passage of acid gastric contents into the duodenum, with consequent stimulation of the secretin mechanism. Instillation of HCl into the duodenum had a similar effect. All subsequent observations were made after a 14 to 16-hour fast, which diminished but did not eliminate these fluctuations. However, the hourly flow and pigment output were quite constant under these circumstances in individual experiments. No satisfactory explanation can be offered for the variation in bile flow and pigment output on different days. This observation is not in accord with that of Kocour and Ivy (8), who reported that in external fistula dogs the bile volume output is constant ( $\pm 4$  per cent) and reproducible under similar experimental conditions. It may be significant in this connection that the Rous-McMaster type fistula dog (9), which they used, has been found to have, almost invariably, some degree of impairment of liver function even though apparently healthy (10, 3). Single or continuous intravenous injections of 0.85 per cent NaCl or one per cent  $\text{Na}_2\text{CO}_3$  solutions, in quantities employed for injection of bilirubin or bromsulfalein, had no significant effect on bile volume or pigment excretion.

**Bilirubin injection.** Four dogs were given eight single intravenous injections of one mg. bilirubin per kg. body weight (in one minute). The pertinent data are

presented in table 2. After a single priming dose (0.3-1.0 mg/kg.), bilirubin was injected continuously for 30 to 120 minutes in 3 dogs (6 experiments).

The injection produced no significant change in the rate of bile flow. The maximum concentration of pigment in the bile ranged from 268 to 433 mg/100 cc. after single injection and from 251 to 727 mg/100 cc. after prolonged injection, occurring in the second 15-minute bile sample in one instance, in the third sample in five and in the fourth sample in eight instances. The period of maximum pigment output coincided with that of maximum concentration in all but five instances, occurring in the preceding 15-minute period in two and in the succeeding 15-minute period in three instances. No significant amount of bilirubin was found in the urine obtained by catheterization at the end of the experimental period. These data do not represent the actual conditions of excretion of pigment by the hepatic cells

TABLE 2. PIGMENT EXCRETION IN BILE FOLLOWING SINGLE INTRAVENOUS INJECTION OF 1 MG. BILIRUBIN PER KILOGRAM BODY WEIGHT

DOG	BILIRUBIN INJECTED	POST-INJECTION MAXIMUM CONCENTRATION (15 MIN. PERIOD)				PIGMENT EXCRETION				RECOVERY			
						Control	Post-injection						
		mg. %	total	vol- ume	time			1st hr.	2nd hr.	3rd hr.	1st hr.	2d hr.	3d hr.
	mg.		mg.	cc.	min.	mg/hr.	mg.	mg.	mg.	%	%	%	%
4	14.8	320	1.60	0.5	60	2.411	6.658	7.397	3.218	28.7	33.7	5.4	67.8
	14.8	268	4.29	1.6	45	2.416	9.074	6.777	3.385	44.0	29.5	6.5	89.0
6	20.0	410	3.28	0.8	60	3.845	10.400	9.772	3.625	32.8	29.6		62.4
	20.0	343	6.52	1.9	30	4.279	15.192	7.279		54.6	15		69.6
8	12.0	334	5.00	1.5	45	1.347	9.145	2.621	2.799	65.0	10.6	12.1	87.7
	12.0	433	1.95	0.45	60	1.333	4.645	5.884	1.690	27.6	37.9	3.0	68.5
	12.0	271	4.07	1.5	60	2.600	6.880	3.393	3.398	35.7	6.6	6.6	48.9
9	14.0	317	3.49	1.1	45	3.778	7.669	7.166	4.901	27.8	24.2	8.0	60.0

because of the relatively low 15-minute bile volume (<2 cc.) as compared to the capacity of the bile duct system. The latter was found to be about 5 cc. in one cholecystectomized, Thomas-type fistula dog terminated six months after operation. The data obtained therefore represent the result of some degree of dilution of freshly secreted bile by the contents of the duct system; there is also a time lag incident to the existence of this 'dead space'.

The percentage recovery of the injected bilirubin was calculated by subtracting the hourly pigment excretion during the pre-injection control period from the subsequent hourly excretion. The accuracy of these recovery values depends upon the validity of the assumption that the basal pigment excretion remains constant during the experimental period. Although this assumption is open to some question, the comparative consistency of hourly pigment excretion in control experiments suggests that this method of calculation is justifiable.

After a single injection of one mg/kg., 48.9 to 89.0 per cent of the amount

administered was recovered in three hours, the largest proportion during the first hour in six instances and during the second hour in two instances (table 2). In the continuous injection experiments (1.3-5.0 mg/kg.), 61.0 to 100 per cent of the quantity administered was recovered within two to four hours. There was no consistent relationship between the percentage recovered and the quantity administered. Some increase in pigment concentration and/or output was almost invariably present in the first 15-minute bile sample and was pronounced in the second 15-minute sample. Interpreted in the light of conditions incident to the existence of the relatively considerable dead space of the bile ducts, these data indicate that an increase in circulating bilirubin results in a prompt increase in the rate of pigment excretion by the hepatic cells.

As pointed out by Greene and Snell (11), this augmented excretion is accomplished primarily by an increase in concentration of pigment in the bile rather than by an increase in bile volume. However, they found no increase in bilirubin excretion during the first 30 minutes after intravenous injection of one mg/kg., the maximum concentration and excretion occurring during the third hour. This discrepancy may be due to the fact that their experiment was conducted under amytal anesthesia, the rate of bile flow being considerably lower than in our dogs. Berman, Snapp and Ivy (12) determined the rate of biliary excretion of bilirubin after injecting 5 mg/kg. in 5 dogs under sodium pentobarbital anesthesia. An average of 29 per cent of the injected bilirubin was recovered in 1.5 hours and 39.4 per cent in 3 hours, the maximum recovery occurring during the second half-hour period. It is doubtful that the greater recovery in our experiments was due to the smaller quantity administered as a single injection (1 mg/kg), inasmuch as approximately the same proportion was recovered by us after prolonged injection of larger amounts. Moreover, Berman *et al.* (12) obtained substantially the same percentage recovery after injection of 12 mg/kg. as after 5 mg/kg. It appears more probable that the lower figures obtained by them are due to the effect of the anesthetic and operative procedure and, possibly, to the fact that we determined the total bile pigment output whereas they determined only bilirubin, by the method of Thannhauser and Anderson (13), which does not give entirely reliable results for pigment in bile.

*Bromsulfalein injection.* Five dogs were given 11 single intravenous injections of 5 mg. of bromsulfalein (BSP) per kg. in one minute. The pertinent data are presented in table 3. After a single priming dose (1 mg/kg.), BSP was injected continuously for two hours in 4 dogs (6 experiments).

There was no significant change in the rate of bile flow. The maximum concentration of dye in the bile ranged from 292 to 1432 mg/100 cc. after single injection and from 278 to 1420 mg/100 cc. after prolonged injection, occurring in the second 15-minute bile sample in four instances in the third sample in five, in the fourth sample in four, in the fifth sample in two, in the sixth sample in two and as late as the seventh sample (105 minutes) in one. It is interesting that in this case (*dog 5*, table 3), the maximum dye output occurred during the second 15-minute period. There was no such close time correspondence between maximum output and maximum concentration of BSP as existed in the case of bile pigment.

The percentage recovery of BSP was of the same order of magnitude as that of

injected bilirubin, viz., 60 to 96.9 per cent in 3 hours after single injection and 48 to 74.7 per cent within 4 hours after beginning a continuous two-hour injection. Dye appeared in the bile within the first 15 minutes in every instance, 41.9 to 69.8 per cent being excreted during the first hour after a single injection. These values are in accord with previous reports from this laboratory (14, 3). What was said above regarding the promptness of biliary excretion of bile pigment apparently applies equally to biliary excretion of bromsulfalein.

There was no evidence that the excretion of pigment was depressed during the period of maximal excretion of BSP. In fact, the amount of pigment excreted during this period exceeded the control level in every instance but one (table 3, dog 7).

TABLE 3. BILIARY EXCRETION OF BSP AND PIGMENT FOLLOWING SINGLE INTRAVENOUS INJECTION OF BSP

DOG	BROMSULFALEIN														PIGMENT EXCRETION			
	Injected		Recovered				Maximum concentration (15-min. periods)				Maximum output (15-min. periods)							
	mg/ kg.	total	1st hr.	2nd hr.	3rd hr.	total	mg. %	total	vol.	time	total	mg. %	vol.	time	control	1st hr.	2nd hr.	3rd hr.
		mg.	%	%	%	%		mg.	cc.	min.	mg.		cc.	min.	mg.	mg.	mg.	mg.
4	5	75	52.5	11.3	1.6	65.4	816	17.1	2.1	30	17.1	876	2.1	30	1.373	2.936	2.767	1.526
	5	75	60.4	11.4	2.2	74.0	864	18.1	2.1	45	18.1	864	2.1	45	2.132	2.742	3.062	2.192
	5	75	59.8	11.7	1.9	73.6	790	18.9	2.4	30	18.9	790	2.4	30	1.604	2.178	2.495	2.783
5	5	90	69.8	17.3	9.8	96.9	1280	6.4	0.5	115	24.7	476	5.2	30	1.784	5.118	1.550	3.312
	5	90	55.9	10.1	3.2	69.2	1432	18.6	1.3	60	22.2	1169	1.9	45	4.076	5.037	0.835	1.089
6	5	100	48.2	13.0	2.2	63.5	974	10.7	1.1	60	19.8	903	2.2	30	3.202	3.316	1.810	0.554
	5	100	41.9	15.3	2.8	60.0	750	1.5	0.2	75	11.7	405	2.9	30	3.005	3.768	2.179	3.678
	5	100	42.0	7.4			673	22.8	3.4	45	22.8	673	3.4	45	3.928	4.925	1.250	
	5	100	46.3	7.6	4.3	64.2	1110	11.1	1.0	60	22.9	1044	2.2	45	4.111	4.965	1.573	3.702
7	5	60	49.3	19.8	1.8	70.9	923	12.9	1.4	45	12.9	923	1.4	45	3.475	2.949	3.674	2.725
9	5	70	41.7	11.3	2.6	55.6	292	8.7	3.0	45	11.7	274	4.3	30	3.061	3.587	4.008	4.359

There is, therefore, no indication of competition between BSP and bilirubin for a common excretory mechanism at normal serum bilirubin concentrations.

*Simultaneous bilirubin and bromsulfalein injection.* After a single priming dose, bilirubin and BSP were injected simultaneously continuously for 90 to 120 minutes. The pertinent data are presented in table 4. In only one case (dog 10) was the period of maximum output of pigment and dye delayed as compared with the findings after individual injections of bilirubin and BSP. Approximately the same proportion of dye was recovered (50.1-96.1 per cent) as after prolonged injection of BSP alone (48-74.7 per cent). However, the proportion of injected bilirubin recovered as biliary pigment (40.9-52.5 per cent) was lower than when bilirubin alone was injected (61-100 per cent). This suggests that, at elevated serum bilirubin concentrations,



bilirubin and BSP may compete for a common excretory mechanism, the BSP being excreted preferentially under these circumstances.

Dragstedt and Mills (15) reported that intravenous injection of bilirubin interfered with removal of BSP from the blood. The data presented here (also table 5) indicate that artificially induced hyperbilirubinemia and increased hepatic excretion of bile pigment do not retard the excretion of BSP in the bile. It is known moreover, that the rate of removal of BSP from the blood may be normal in uncomplicated clinical or experimental hemolytic jaundice.

The data presented in table 5 indicate the quantitative discrepancy between the rate of removal of injected bilirubin and BSP from the circulating plasma and the rate of their excretion in the bile. The total quantity in the plasma was estimated on

TABLE 4. BILIARY EXCRETION OF BSP AND PIGMENT DURING AND AFTER PROLONGED INTRAVENOUS INJECTION OF BSP AND BILIRUBIN

DOG	PIGMENT + DYE INJECT.		TEST PERIOD		RECOVERY		MAXIMUM CONCENTRATION (15-MIN. PERIOD)				MAXIMUM OUTPUT (15-min. period)			
	mg/ kg.	total	injec- tion	post- injec- tion	during injec- tion	total	mg. %	total	vol.	time	total	mg. %	vol.	time
Bromsulfalein														
		mg.	min.	min.	%	%		mg.	cc.	min.	mg.		cc.	min.
1	5.5	55.2	120	120	30.1	55.6	642	1.99	0.31	180	4.68	624	0.75	75
2	4.8	48.2	90	120	49.3	73.8	200	5.8	2.9	45				
3	5.2	110	120	120	36.9	50.1	178	7.48	4.2	45	8.33	167	5.0	30
10	5.0	74.6	120	105	70.1	96.1	805	7.84	1.8	120				
Pigment														
1	5.5	55.2	120	120	22.7	41.4	684	2.12	0.31	180	3.95	527	0.75	75
2	4.8	48.2	90	120	38.1	52.5	150	4.35	2.9	45				
3	5.2	110	120	120	26.6	40.9	151	7.53	5.0	30				
10	5.0	74.6	120	105	28.7	41.3	530	1.13	0.2	195	7.84	435	1.8	120

the basis of 50 cc. of plasma per kilogram of body weight. At the end of a two-hour injection period, 43 to 71 per cent of the bilirubin and 34.6 to 63 per cent of the BSP had been removed from the blood stream but had not been excreted in the bile. In no case was a significant amount of either detected in the urine during the period of the experiment. Even if one assumes a distribution of these substances throughout the extracellular fluid at their plasma concentrations, which is highly improbable (7), and also takes into consideration the 'dead space' of the bile duct system, a considerable fraction of the injected pigment and dye remains unaccounted for. If the liver is the only organ involved in their removal from the blood, this implies a subsequent phase of temporary storage in that organ prior to their passage into the bile. It has been suggested (16-19) that the reticuloendothelial system may be involved in the removal of BSP from the blood. There is no clear evidence, however, that extra-hepatic tissues are involved significantly in the removal of bilirubin from the blood. The data presented here suggest that these substances are excreted in essentially the

same manner and, when introduced into the circulation, are removed from the body rather promptly, and in large amounts, by the liver. In the light of these findings,

TABLE 5. PERCENTAGES OF BSP AND BILIRUBIN REMOVED FROM THE BLOOD BUT NOT EXCRETED IN THE BILE DURING INTRAVENOUS INJECTION OF BSP AND BILIRUBIN

DOG	WT. kg.	TIME hr.	BILIRUBIN								BROMSULFALEIN							
			Amt. Injected		Excreted		Circulating		Removed, not excreted		Amt. injected		Excreted		Circulating		Removed, not excreted	
			mg.		mg.	%	mg.	%	mg.	%	mg.		mg.	%	mg.	%	mg.	%
1	9	$\frac{1}{2}$	24.75		3.97	16	2.25	9.0	18.5	75								
		1	31.50		11.64	37	2.25	7.0	17.6	56								
		$1\frac{1}{2}$	38.25		18.26	48	2.25	6.0	16.7	46								
		2	45		23.7	53	2.00	4.0	19.3	43								
3	21	$\frac{1}{2}$									128	26.5	20.7	4.2	3.3	97.3	76	
		1									168	69.2	41.2	4.4	2.8	94.4	56	
		$1\frac{1}{2}$									208	99.7	47.9	4.2	2.1	104.1	50	
		2									253	130.7	51.6	3.7	1.4	118.6	47	
4	15	$\frac{1}{2}$									43.6	8.0	18.3	2.0	4.7	33.6	77	
		1									57.2	24.6	43	2.0	2.5	30.6	53.5	
		$1\frac{1}{2}$									70.8	36.5	51.5	2.0	2.9	32.3	45.6	
		2									84.6	45.5	53.8	2.0	2.3	37.1	43.9	
4	15	$\frac{1}{2}$									85.5	23	26.9	4.5	5.3	58	67.8	
		1									111.0	64.5	58.2	4.0	3.5	42.5	38.3	
		$1\frac{1}{2}$									136.5	84.2	61.7	3.75	3.1	48	35.2	
		2									162.0	103.4	63.8	2.6	1.6	56	34.6	
2	10	$\frac{1}{2}$									26.25	2.55	9.7	2.0	7.3	21.7	83	
		1									32.5	8.8	27.1	2.0	6.9	21.7	66	
		$1\frac{1}{2}$									38.75	13.15	33.9	2.0	5.1	23.6	61	
		2									45.0	26.1	58	2.0	4.0	16.9	38	
1	10	$\frac{1}{2}$	27.3	0.82	3	3	11.0	23.5	86		27.3	0.33	1.2	2	7.3	25	91.5	
		1	36.6	3.5	9.8	3.5	9.2	29.6	81		36.6	4.6	12.6	2	5.4	30	82	
		$1\frac{1}{2}$	45.9	9.7	21	3	7.0	33.2	72		45.9	13.1	28.5	2	8.5	30.8	63	
		2	55.2	12	22	3	7.0	39	71		55.2	16.7	30.2	2	6.8	35	63	
3	21	$\frac{1}{2}$	59	7.9	13	4.5	8.0	46.5	79		59	8.66	14.6	2.6	4.9	47.5	80.5	
		1	76	16.56	22	5	6.0	54.5	72		76	22.23	29.2	2.6	3.8	51	67	
		$1\frac{1}{2}$	93	23.41	25	3.6	4.0	66	71		93	32.34	34.7	0.1	0.3	60.5	65	
		2	110	30	27	4.6	6.5	73	66.5		110	40.6	36.9	2.6	4.1	65	59	

TABLE 6. BILIARY EXCRETION OF PIGMENT DURING AND AFTER INTRAVENOUS INJECTION OF LAKED RED BLOOD CELLS

DOG	CONTROL PERIOD			PACKED CELLS INJECTED	INJECTION PERIOD (60 MIN.)			POST-INJECTION PERIOD							
	Pigment excretion				Pigment excretion			First hour			Second hour				
	vol.		pigment		vol.		pigment	vol.		pigment		vol.		pigment	
	cc/hr.	mg. %	mg/hr.		cc.	cc/hr.	mg. %	mg/hr.	cc/hr.	mg. %	mg/hr.	cc/hr.	mg. %	mg/hr.	
9	3.4	37.6	1.404	50	4.7	65.5	3.173	8.8	235.7	20.693	10.2	285.6	28.246		
	5.3	37.2	1.931	45	7.0	68.6	4.918	10.9	238.0	26.518	11.8	321.6	38.509		

the observation of Cohn *et al.* (20) that the peripheral tissues are capable of removing considerable amounts of BSP from the blood in the absence of the liver cannot be

interpreted as indicative of the importance of extrahepatic tissues in this connection in the intact animal.

*Hemoglobin injection.* Fifty cc. and 45 cc. of packed red blood cells were laked and injected intravenously at an interval of two months. The pertinent data are presented in table 6. The content of bilirubin-precursor (protoporphyrin) in these quantities of laked cells was approximately 600 mg. and 540 mg., respectively. The volume of bile increased steadily during the 3-hour experimental period. A definite increase in pigment excretion occurred in the fourth 15-minute sample in the first experiment and in the third sample in the second experiment. Both the concentration and total quantity of pigment in the bile increased progressively during the experimental period. A similar observation was reported by Greene and Snell (11) in an acute experiment. These data emphasize the rapidity of transformation of intravenously injected hemoglobin to bilirubin and the promptness of excretion of the latter in the bile. In view of the magnitude of the biliary 'dead space' (about 5 cc.) in relation to the 15-minute bile volume (1.4–1.9 cc. during the injection period), it is apparent that these phenomena must be initiated almost immediately after the introduction of hemoglobin into the circulation.

#### SUMMARY

Determinations were made of the rate of bile flow and pigment excretion in trained, Thomas-type tubulated duodenal-fistula dogs, bile being obtained by inserting a temporary glass cannula into the common duct through the duodenal opening. There was considerable daily variation in the rate of bile flow and of pigment excretion in untreated animals.

After both single and prolonged intravenous injections of bilirubin, there was a prompt increase in the rate of pigment excretion in the bile, accomplished chiefly by an increase in concentration. After a single injection of one mg/kg., 48.9 to 89 per cent of the amount administered was excreted in the bile in three hours, the largest proportion usually in the first hour. Sixty-one to one hundred per cent of the quantity administered by prolonged injection was excreted in four hours.

Bromosulfalein, injected intravenously, appeared promptly in the bile and was recovered in approximately the same proportions as was bilirubin. Pigment excretion was not depressed during the period of maximal excretion of BSP.

When bilirubin and BSP were injected simultaneously, BSP was excreted as efficiently as when administered alone, but the proportion of injected bilirubin recovered as biliary pigment was lower than when bilirubin alone was injected. This suggests that, at elevated serum bilirubin concentrations, bilirubin and BSP may compete for a common excretory mechanism, the BSP being excreted preferentially.

The quantity of bilirubin and BSP removed from the blood stream at any time during prolonged injection greatly exceeded the quantity excreted in the bile up to that time. If the liver is the only organ involved in the removal of these substances from the blood, this implies a phase of temporary storage in that organ prior to their passage into the bile.

Intravenous injection of laked red blood cells resulted in a prompt increase in

pigment excretion in the bile, indicating the rapidity of transformation of hemo-globin to bilirubin under these circumstances.

The authors are indebted to Dr. J. Earl Thomas for advice and assistance in the preparation of the dogs used in these studies.

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# RENAL FUNCTION IN NORMAL RABBITS AND DOGS AND THE EFFECT OF URANYL SALTS<sup>1</sup>

J. HENRY WILLS<sup>2</sup> AND EDNA MAIN

*From the Division of Pharmacology and Toxicology, Department of Radiation Biology of the University of Rochester, School of Medicine and Dentistry*

ROCHESTER, NEW YORK

IT HAS been shown by MacNider (1) that the kidney is the organ affected predominantly by administration of uranium to animals. Accordingly, some investigations of the renal action of uranium were carried out as part of an extensive study of the effects of this metal on mammals. This paper is confined to the effects of uranium on the handling by the kidney of chloride, inulin and diodrast—the three substances with which most work was done.

## METHODS

Exposures to inhalation of uraniferous dusts were carried out in closed chambers by methods to be described elsewhere (2). In general, the entire animal was exposed to the dusty atmosphere of approximately constant composition for roughly six hours a day on six days of the week. Duration of inhalation exposure is stated in total hours. Animals injected with uranyl acetate received intravenous doses of a 0.3 per cent aqueous solution.

*Clearance Determinations—Rabbit.* The animal was anesthetized with nembutal (Veterinary Solution), the dose being derived from a curve similar to that of Bazett and Erb (3) but shifted downward so that a rabbit of 3-kg. body weight received 1.8 ml. of solution. Additional anesthetic was given as required to maintain an approximate plateau of anesthesia (injection of 0.2 ml. of solution every 30 min. usually had the desired effect on a 2.5 kg. rabbit).

Cannulas were tied into the trachea, the left saphenous vein and the bladder in that order. As soon as the venous cannula had been inserted, infusion of the animal with a saline solution containing inulin (0.25%) and diodrast (0.52 vol.% of a 30% solution) was started at the rate of 25 ml/kg/30 min. After priming for 30 minutes, the infusion rate was lowered to one ml/min. for the duration of the experiment.

After an equilibration period of at least one hour from the end of priming, collection of successive urine samples at 30-minute intervals was started. Arterial blood samples were taken from one deep femoral artery at the midpoints of the periods of urine collection. At least two determinations were made on each animal.

Table 1 contains sample data to illustrate the rabbit experiments. It shows

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Received for publication June 25, 1948.

<sup>1</sup> This paper is based on work performed under contract with the Manhattan District (U. S. Engineers' Office) at the University of Rochester, Rochester, N. Y.

<sup>2</sup> Present address: Medical Division, Army Chemical Center, Maryland.

that we were able to maintain fairly constant blood levels of the substances studied and that in the control experiments urine flow was fairly constant for the most part. After injection of uranyl acetate, the urine rate became more variable as a result of the action of the metal on the nephron.

*Clearance Determinations—Dog.* Females were used, the animal lying on its side on a table. Restraint ties were used only when necessary. The dog was catheterized with a soft-rubber, wing-tip catheter and a hypodermic needle was taped in place in one leg vein.

The dog was primed by intravenous infusion for 30 minutes at the rate of 3 ml/min. with a sterile saline solution containing inulin (0.7%) and diodrast (1.4

TABLE 1. DATA FROM TYPICAL CONTROL AND POISONED RABBITS OBTAINED DURING THE STUDY OF THE ACUTE EFFECTS OF INTRAVENOUS INJECTION OF URANYL ACETATE

TIME	PLASMA CHLORIDE	PLASMA DIODRAST	PLASMA INULIN	URINE CHLORIDE	URINE DIODRAST	URINE INULIN	URINE RATE	CLEAR- ANCE CHLORIDE	CLEAR- ANCE DIODRAST	CLEAR- ANCE INULIN
15/12/44 Control (3.75 kg.)										
	mg. %	mg. I %	mg. %	mg. %	mg. I %	mg. %	ml/min.	ml/min.	ml/min.	ml/min.
1:00-1:30	420	1.92	21.0	1007	510	1320	0.29	0.70	77.1	18.4
1:30-2:00	422	1.92	21.1	850	352	880	0.32	0.64	58.7	13.3
2:00-2:30	427	1.93	21.3	773	350	880	0.34	0.62	61.6	14.2
2:30-3:00	433	1.92	21.1	850	447	1120	0.28	0.55	65.2	14.9
3:00-3:30	440	1.86	20.5	898	385	944	0.32	0.65	66.4	14.7
3:30-4:00	444	1.81	20.2	510	169	420	0.66	0.76	61.6	13.8
12/2/45 (2.56 kg.)										
1:00-1:30	416	6.07	40.2	410	126	264	2.00	1.97	41.5	13.1
1:30	3 mg./kg. UO <sub>2</sub> Ac <sub>2</sub> I.V.									
1:30-2:00	430	6.10	42.2	438	158	320	1.12	1.14	29.0	8.5
2:00-2:30	430	6.00	42.8	481	124	320	1.58	1.77	32.7	11.9
2:30-3:00	431	5.94	43.3	469	120	292	1.67	1.81	33.8	11.2
3:00-3:30	431	5.84	43.8	460	139	332	1.36	1.45	32.4	10.3
3:30-4:00	428	5.85	43.9	491	124	325	1.61	1.85	34.2	11.9
4:00-4:30	426	5.90	44.1	467	136	328	1.42	1.56	32.8	10.6
4:30-5:00	423	5.83	44.2	467	128	332	1.43	1.58	31.4	10.7

vol. % of a 35% solution). At the end of the priming period, infusion was continued at the same rate but with a solution containing half the above concentrations of inulin and diodrast. Equilibration was allowed for at least an hour after the priming period before starting collection of clearance samples. Four consecutive urine samples were collected at 15-minute intervals, the bladder being washed out with 10 ml. of warm, sterile-distilled water at the end of each period. A venous blood sample was taken at the midpoint of each period.

The dog experiments were carried out under our direction by Drs. J. Roberts, D. C. Brodie, D. L. Adler and Mr. J. Tournaben. The chemical analyses of the samples and the analysis of the results were made by ourselves.

## ANALYTICAL METHODS

Urine and oxalated plasma samples were analyzed for chloride by methods of Peters and Van Slyke (4, pp. 833-834). Their inulin concentrations were estimated by the method of Harrison (5). Fermentable reducing substances in both urine and plasma were separated from the non-fermentable by the method of Van Slyke and Hawkins (6).

Urine and whole blood were analyzed for diodrast by the method of Alpert (7). According to Bobey and Price (8), appreciable amounts of diodrast are held by the precipitated red cells when whole dog blood is analyzed by this method. This observation has been checked by us and extended to the rabbit. In our work it was found that the plasma diodrast level could be calculated from the whole blood analysis for the dog by equation 1 and for the rabbit by equation 2.

$$P_D = \frac{100W_D}{90.6 - 46.7V_c + 5.8V_c^2} \quad 1$$

$$P_D = \frac{100W_D}{96.6 - 93.4V_c + 6.6V_c^2} \quad 2$$

Here  $P_D$  is the plasma concentration of diodrast in mg. per cent,  $W_D$  is the whole blood concentration of diodrast and  $V_c$  is the cell fraction determined by hematocrit.

*Interpretation of Data.* The analytical data and the figures for rate of urine flow were used for calculation of urinary clearances in ml/min. As a measure of tubular function we have used the ratio of the amount of substance transferred across the tubular membrane to that transferred across the glomerular one. For reabsorbed substances, like chloride, this function is calculated by equation 3.

$$f_{Cl} = 1 - \frac{U_{Cl}P_{In}}{U_{In}P_{Cl}} \quad 3$$

while for secreted materials, like diodrast, equation 4 is used

$$f_D = \frac{U_D P_{In}}{U_{In} P_D} - 1 \quad 4$$

Here  $U$  is the urinary concentration,  $P$  is the plasma concentration and the subscripts Cl, D and In refer respectively to chloride, diodrast and inulin.

Chloride has been found to be reabsorbed partially in the proximal convolution and partly in more distal portions of the nephron (9). Since filtered chloride is reabsorbed almost completely, a decrease in the  $f_{Cl}$  would be a fairly sensitive indicator of interference with tubular function but would not tell anything about the locus of the action.

On the other hand, a decrease in the  $f_D$  indicates not only interference with tubular function but, also, to some extent the locus of the action. Since phlorizin lowers the ability of the tubule to transport diodrast (10) and since the tubular transports of glucose and diodrast are affected similarly by thyroxin (11), there is a good probability that diodrast is secreted at least in part in the same portion of the nephron as is concerned with reabsorption of glucose. Glucose is stated to be reabsorbed almost completely in the first half of the proximal convolution (9). Thus, a decrease in  $f_D$  is believed to indicate impaired function in the proximal segment.

The possibility of change in the physical properties of the nephron, within a few days after poisoning with uranyl salts (12), makes it advisable to use only immediate effects on renal function in attempting to identify the locus of uranium action on the kidney. The same factor makes values of the filtration fraction of doubtful significance some days after the injection of uranium salt.

## RESULTS

*Renal Function in Normal Rabbits and Dogs.* In normal animals, all the measures of renal function studied here appeared to be correlated to some extent with the rate

TABLE 2. EQUATIONS OF LEAST SQUARE LINES EXPRESSING THE COVARIANCES OF URINE RATE AND THE VALUES OF VARIOUS RENAL FUNCTIONS IN CONTROL ANIMALS

FUNCTION	RABBIT		DOG	
	Equation	No. of Points	Equation	No. of Points
CCl	$0.04 + 1.08u$	48	$0.73u - 0.31$	61
C <sub>D</sub>	$50.6 + 13.7u$	29	$212 - 0.67u$	62
C <sub>In</sub>	$12.6 + 0.91u$	45	$53.6 + 3.0u$	54
f <sub>Cl</sub>	$1.00 - 0.07u$	23	$1.00 - 0.01u$	51
f <sub>D</sub>	$2.02 + 0.66u$	28	$2.96 - 0.16u$	52

Clearances (C) in ml/min.  $u$  = urine rate in ml/min.  $f$  = tubular transport per unit of filtration, defined by text equations 3 and 4.

TABLE 3. EXAMINATION OF THE SIGNIFICANCE OF THE LEAST SQUARE LINES OF TABLE 2.

FUNCTION	RABBIT						DOG					
	S <sub>y</sub>	u <sub>min.</sub>	X <sub>1</sub>	u <sub>max.</sub>	X <sub>2</sub>	$\frac{X_2 - X_1}{S_y}$	S <sub>y</sub>	u <sub>min.</sub>	X <sub>1</sub>	u <sub>max.</sub>	X <sub>2</sub>	$\frac{X_2 - X_1}{S_y}$
CCl	0.22	0.04	0.08	2.42	2.66	11.7	0.47	1.3	0.68	9.5	6.58	12.5
C <sub>D</sub>	20.3	0.05	51.0	2.42	84.0	1.6	50	1.3	211	9.5	205	0.1
C <sub>In</sub>	4.8	0.02	12.6	2.42	14.9	0.5	8.9	1.3	57.5	9.5	81.8	2.7
f <sub>Cl</sub>	0.04	0.04	0.99	2.42	0.83	4.0	0.01	1.3	0.99	9.5	0.92	7.0
f <sub>D</sub>	1.05	0.16	3.13	2.42	4.60	1.4	0.64	1.3	2.75	9.5	1.48	2.0

S<sub>y</sub> = standard error of estimate.  $u_{min}$  and  $u_{max}$  = minimal and maximal experimental rates of urine flow, in ml/min.  $X_1$  and  $X_2$  = values on regression line corresponding respectively to  $u_{min}$  and  $u_{max}$ .

of urine flow. Table 2 gives the equations of the least square lines for the relations between the various parameters and the urinary rate in our experiments. The lines of table 2 were examined for significance by the following steps: *a*) calculation of the standard error of estimate (S<sub>y</sub>), *b*) calculation of the difference between the regression values at the extreme experimental urine flows and *c*) calculation of the ratio of the value found in *b* to that found in *a*. The results of this process are given in table 3.

It is seen from table 3 that in our experiments the only variables having clearly significant correlations with urine flow are the clearance of chloride and the reabsorption of chloride. This holds for both the dog and the rabbit, although in the dog there appears to be a fair chance (143:1) that the inulin clearance also is a function



of the rate of urine flow. The latter finding is of interest in that it shows a clear difference between our series of experiments and that of Shannon (13) in which the latter author found no relationship between glomerular filtration in the dog and the rate of urine flow. Our data on inulin clearance in the rabbit differ from those of Kaplan and Smith (14) in that we found no significant relationship between the clearance and the urine rate while Kaplan and Smith thought that there was such a correlation. The reason for our unorthodox results with inulin clearance is not apparent.

Although the relation of most of our parameters to urine rate was not of high order, it appeared that it would be more correct to compare our experimental values to those obtained from these regression lines than to compare them to a mean normal value. Accordingly, in assessing the effects of administration of uranium compounds upon renal function, the appropriate lines of table 2 were used to evaluate the expected levels of the variables at the experimental rates of urine flow. The experimentally determined values of the parameters were then compared with the expected ones.

*Acute Effects of Intravenous Injection of Uranyl Acetate in the Rabbit.* The typical response of urine flow to intravenous injection of uranyl acetate was an immediate increase followed by a slow decrease. The urine rate fell from its peak more rapidly with high doses than with small ones. The upper curve of figure 1a, in which the urine rate at 2 hours 10 minutes after the injection (expressed in percentage of the control rate) is plotted against the dose of uranyl acetate, shows that the maximal increase in urine flow is produced by a dose of about 0.5 mg/kg. of the uranium salt.

For comparison with our curve there is drawn on this figure a graph derived from data of Watanabe, Oliver and Addis (15) showing the effects of various doses of uranyl acetate upon the secretion of urine 72 hours after administration of the salt. These two graphs show that the dose of uranyl acetate which produced the maximal acceleration of urine flow in our short-time experiments also produced some acceleration of urine rate 72 hours after the injection. Doses of the uranyl salt greater than 1 mg/kg. produced some increase in urine flow 2.17 hours after the injection, but produced oliguria or anuria within three days.

The acute effects (2.17 hours after the injection) of intravenous injections of uranyl acetate upon renal clearances are shown in figures 1b, 1c and 1d. The only clearly significant change shown here was a decrease in the diodrast clearance.

The decreased clearance of diodrast could be the result of decreased blood flow through the kidney but this is believed not to be the case. Unpublished work of D. L. Adler in this laboratory has shown that intravenous injection of uranyl acetate does not decrease blood flow through the frog glomerulus. Direct measurements of blood flow through the mammalian kidney (12, 16) have shown also that injection of uranyl salts does not decrease the circulation through the kidney. This leaves as the alternative explanation for the decrease in diodrast clearance after injection of uranyl acetate some interference with the secretory ability of the tubular epithelium.

Figure 1c shows that there was no immediate diminution in chloride reabsorption although there was in diodrast secretion. These findings suggest that the uranyl salt interfered with the function of the proximal convolution of the nephron.

*Subacute Effects of Inhalation of Uranyl Fluoride by the Rabbit.* Figure 2 shows

the results of renal function studies on rabbits exposed to atmospheres of various concentrations of uranyl fluoride dust for different periods of time. Each point represents a different animal, so that the large peaks in the uppermost curves of figures 2b and 2c probably have no significance.

Figure 2 demonstrates that the clearance of chloride was not affected by any of the dust concentrations used although the clearances of diodrast and inulin were reduced definitely by the largest one. The latter two clearances may have been reduced by the intermediate concentration of dust. The lowered clearance of inulin

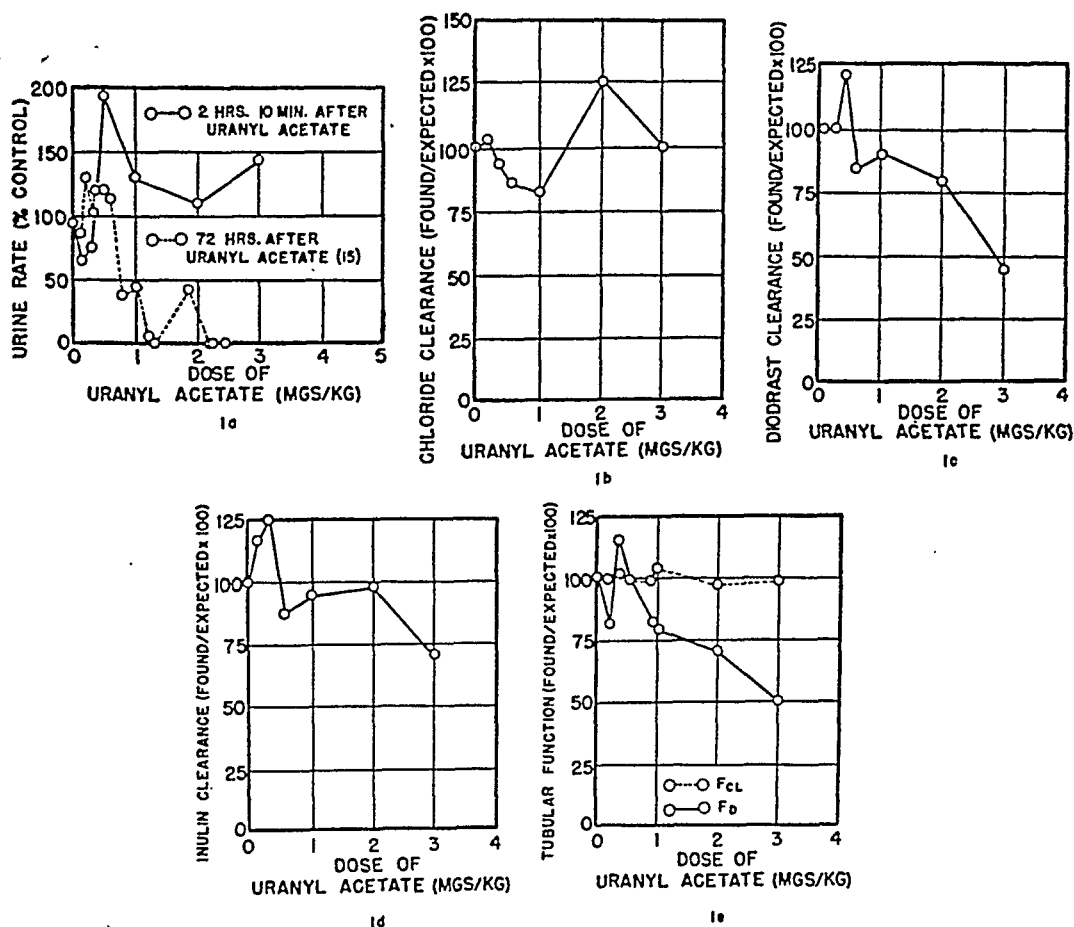


Fig. 1. IMMEDIATE (2 hrs., 10 min.) EFFECTS OF INTRAVENOUS DOSES of uranyl acetate upon the rate of urinary excretion and upon renal function. Each point represents an average of one determination on each of 3 rabbits.

during the dust exposure, particularly marked at the 2.78 mg/M<sup>3</sup> level, does not mean necessarily that there was a decreased rate of filtration through the glomerulus (12). It may mean, rather, that the tubular epithelium had been so altered by the action of the uranium as to be permeable to substances which ordinarily are unable to pass from the tubular lumen into the peritubular capillaries.

When tubular function in these three inhalation experiments was assessed by equations 3 and 4, the curves of figures 2d and 2e were obtained. These graphs show that chloride reabsorption was unaffected except by the highest dust concentration although there appears to have been some effect on diodrast secretion by all dust concentrations. The decrease in diodrast secretion appears to have been lesser in magnitude and later in appearance with the smaller concentrations of uranyl fluoride dust.

It is evident from figure 2 that renal function goes through a minimum during continued exposure to atmospheres containing uranyl fluoride. In nonfatal exposures the maximal effect is produced early in the exposure and is followed by partial recovery of renal function. The extents of effect and recovery and the time courses of these actions vary with the function studied. Thus, it is evident from figures 2d

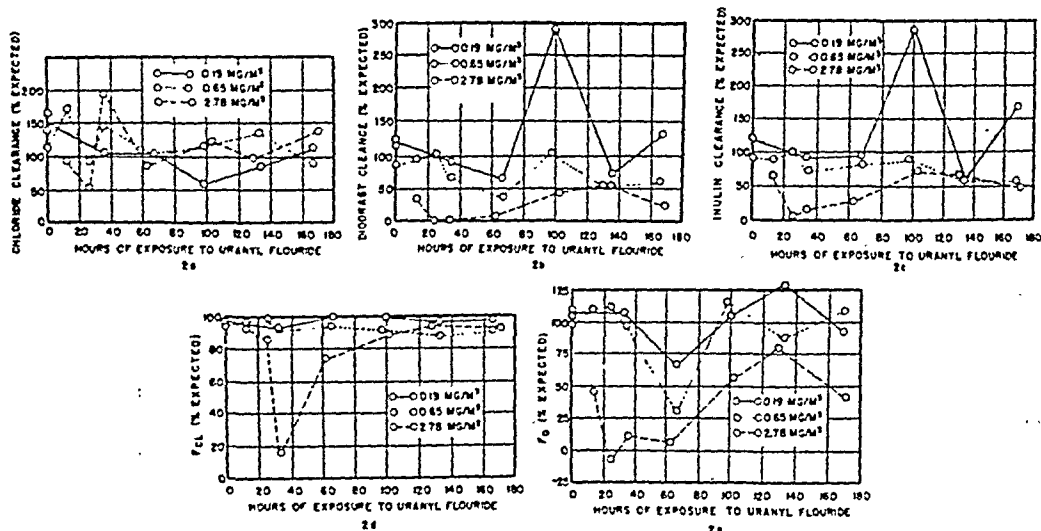


Fig. 2. EFFECTS UPON RENAL FUNCTION of exposures to atmospheres containing various concentrations of uranyl fluoride dust for different lengths of time. Each point represents the mean of two successive determinations upon a single rabbit.

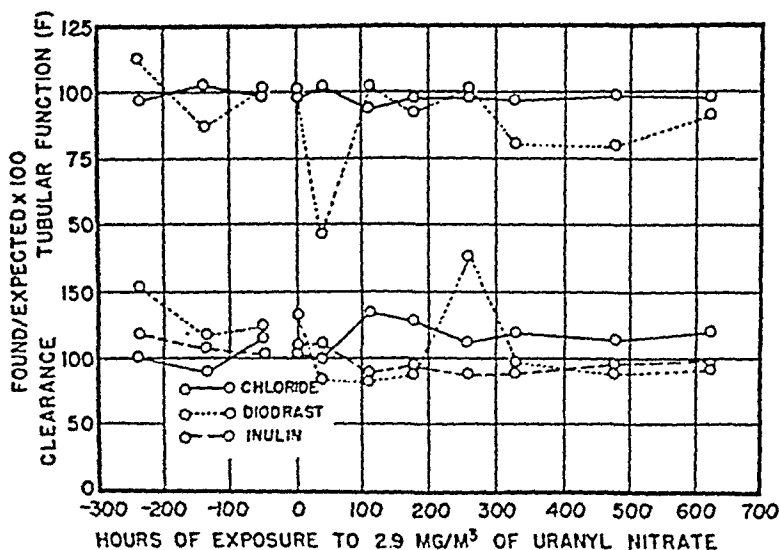


Fig. 3. EFFECT UPON RENAL FUNCTION of dog 695 of exposures to an atmosphere containing 2.9 mg/M³ of a dust of uranyl nitrate. Each point represents the mean of 4 successive determinations.

and 2e that the secretion of diodrast was affected more markedly than the reabsorption of chloride and that the maximal effect on diodrast secretion occurred earlier and persisted longer than that on chloride reabsorption. This temporal difference between the minima in chloride reabsorption and diodrast secretion may mean that these two processes occur by different mechanisms in the tubule.

*Subacute Effects of Inhalation of Uranyl Nitrate by the Dog.* Three dogs were

exposed to an atmosphere containing an average of 0.3 mg/M<sup>3</sup> of uranyl nitrate dust and 2 to one containing an average of 2.9 mg/M<sup>3</sup>. The animals were placed in the dusty atmospheres for about six hours a day, the rest of their time being spent in normal room air.

Figure 3 shows the results on one of the dogs exposed to the high level of dust. It is obvious that in the dog exposed to uranyl nitrate the effects of the exposure are the same qualitatively as in the rabbit exposed to uranyl fluoride. Again it is apparent that the secretion of diodrast is affected more than the reabsorption of chloride and that the clearance of diodrast is the only clearance reduced appreciably by the exposure.

Because the secretion of diodrast appears to be the most sensitive of the measures used here, table 4 summarizes the effects of the two levels of exposure to uranyl nitrate upon  $f_D$  of the dog. It can be seen that the low level of exposure probably had no significant effect on the ability of the tubules to transport diodrast. The high level of exposure, however, had a definite effect in decreasing the ability of the tubule to secrete diodrast.

TABLE 4. EFFECT OF INHALATION OF URANYL NITRATE DUST UPON THE SECRETION OF DIODRAST BY THE TUBULE OF THE DOG

DUST LEVEL	DOG NO.	$f_D$ (FOUND/EXPECTED $\times$ 100)		HRS. OF EXPOSURE FOR MINIMAL $f_D$
		Av. Pre-Exposure	Min. during Exposure	
0.3 mg./M <sup>3</sup>	575	95	76.8	36 and 468
0.3 mg./M <sup>3</sup>	636	99	83.2	258
0.3 mg./M <sup>3</sup>	666	101	81.0	468
2.9 mg./M <sup>3</sup>	656	71	-25.3	48
2.9 mg./M <sup>3</sup>	695	134	47.9	36

#### DISCUSSION

Our findings about renal function in the normal dog and rabbit agree in general with those already in the literature. Our results differ from the literature in the previously discussed finding that inulin clearance in the dog might be correlated with urine rate while in the rabbit it was not. This reversal of the classical situation may depend on our experimental conditions but we are unable to identify the responsible factor.

All of the experiments reported here indicate that the effect of uranyl salts on renal function is the result of interference with the structural and functional integrity of the proximal convolution of the tubules. The fact that reabsorption of chloride is affected only moderately as compared with the secretion of diodrast suggests that the action of uranium is restricted to the proximal convolution rather than affecting both convolutions of the nephron. The microdissection studies of Oliver (17) have localized the histological uranium action in the latter part of the proximal segment.

The negative values of  $f_D$  obtained in some rabbits (fig. 2e) and dogs (dog 656, table 4) indicate that not only has the ability of the tubule to secrete diodrast been removed completely, but also the normal barrier to diffusion of filtered diodrast from the tubular lumen has been breached in part. Comparison of figures 2c and 2e

shows that the only rabbits showing a decreased inulin clearance were the ones with markedly decreased ability to secrete diodrast. This correlation indicates that uranium brought about an increased permeability of the tubular epithelium since it has been pointed out already that probably the blood flow to the kidney, and consequently the filtration, does not change.

#### CONCLUSIONS

1. In the kidney of the normal dog and rabbit the clearance of chloride and the reabsorption of chloride by the tubule are functions of the urine rate; the value of the chloride clearance increases with urine rate and the fractional reabsorption of chloride decreases slightly. The ability of the kidney of the normal dog and rabbit to clear diodrast from the blood by secretion is not a function of urine rate. Under the conditions of our experiments the inulin clearance appears to be a function of urine rate in the dog but not in the rabbit. Uranyl salts affect the function of the proximal convolution, this effect leading to decreased reabsorption of chloride, decreased secretion of diodrast and probably to increased permeability of the tubular membrane to water and dissolved substances.

#### ADDENDUM

Since the completion of this work in June 1946, Lotspeich, Swan and Pitts (18) have studied the renal tubular reabsorption of chloride. They found that in the dog the rate of tubular reabsorption of chloride is a direct, linear function of the rate of glomerular filtration. Their line had a slope of nearly one, agreeing thereby with our findings that the fractional reabsorption of chloride decreases only slightly with increasing urine flow. This slight decrease is significant, however, for both dog and rabbit.

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# UNILATERAL ADRENALECTOMY, UNILATERAL SPLANCHNIC NERVE RESECTION AND HOMOLATERAL RENAL FUNCTION<sup>1</sup>

JOSEPH P. KRISS, PALMER H. FUTCHER, AND MELVIN L. GOLDMAN<sup>2</sup>

*From the Department of Internal Medicine, Washington University School of Medicine*

ST. LOUIS, MISSOURI

IN 1914 Cow (1) reported the anatomical and physiological demonstration of a vascular connection between the adrenal gland and the homolateral renal capsule of the cat. It was postulated that via this communication adrenalin could reach the kidney without first passing through the general circulation. Removal of one adrenal gland resulted in markedly increased production of urine by the kidney on the side operated upon compared with the urine flow on the unoperated side. The blood flow in the kidney homolateral to the excised adrenal was not found increased in proportion to the flow of urine. Cow explained the effects observed as due to the failure of adrenalin to reach the homolateral kidney via the direct adrenal-renal circulation. Marshall and Kolls (2) subsequently observed in dogs an increased excretion of water, chloride and urea by the kidney on the side on which adrenalectomy had been performed, but the simultaneous homolateral excretion of creatinine, phenolsulphonephthalein and lactose was approximately the same or only slightly increased as compared to the normal side. Because similar results were obtained by unilateral section of the splanchnic nerve and by section of the renal nerves, Marshall and Kolls concluded that unilateral removal of the adrenal gland affects the kidney on the same side only in so far as nerves associated with the kidney are injured.

Most evidence (3) favors the concept that the renal nerves have no direct influence on renal tubular reabsorptive or excretory functions. Therefore, it may be presumed that if, as suggested by Marshall and Kolls, injury to the renal nerves is responsible for the effects of adrenalectomy upon excretion of water and solids described above, these effects occur in association with the increased rate of renal blood flow which denervation of the kidney occasions in the anesthetized animal (4-6). However, the methods available to Marshall and Kolls afforded little evidence of a consistently increased rate of blood flow or glomerular filtration as a result of unilateral adrenalectomy. The creatinine clearance has been shown to be a valid measure of glomerular filtration rate in the dog (6, 7) and the phenolsulphonephthalein clearance is roughly correlated with the renal blood flow (6, 8). Thus, the failure of a markedly increased excretion of creatinine or phenolsulphonephthalein to accompany consistently the unilateral diuresis of water and chloride

Received for publication June 14, 1948.

<sup>1</sup> This investigation was supported by a contract with the Office of Naval Research, U. S. Navy Department and a grant from the U. S. Public Health Service.

<sup>2</sup> National Institute of Health Postdoctorate Research Fellow.

observed in the unilaterally adrenalectomized dog (2) suggests that the diuresis observed by Marshall and Kolls was not dependent on differences in *glomerular filtration rate or renal blood flow*. Cow's anatomical and physiological findings, and the subsequent demonstration (9, 10) of the marked influence of the adrenal glands upon the excretion of sodium chloride and water by the kidneys, seem to justify reexamination of Marshall's and Koll's contention that the adrenal gland does not influence specifically the function of the homolateral kidney. Renal clearance techniques afford the opportunity to investigate more thoroughly the effect of unilateral adrenalectomy upon the hemodynamics of each kidney simultaneously and to correlate observed changes with the urinary excretion of water and chloride.

### METHODS

Mongrel dogs maintained on a diet of Friskies Dog Food Cubes (Purina) and occasional feedings of horse meat were used in all experiments.

*Operative procedures.* Intravenous nembutal (.029 gm/kg. body weight) was the anesthetic agent employed during all operations and clearance determinations. Using sterile operative technique, the left adrenal gland was exposed retroperitoneally. The large lumbar vein, which regularly crosses the anterior surface of the adrenal gland to be joined medially by the main adrenal vein, was ligated on both sides of the gland and the adrenal was dissected away from adjacent sympathetic nerves as carefully as possible. Injury to these nerves during dissection seemed inevitable.

Supradiaphragmatic splanchnic section or sympathectomy were performed while intermittent positive pressure was administered by intratracheal catheter. In performing the supradiaphragmatic neurectomies, 2 to 5 cm. of splanchnic nerve ('splanchnic resection') and usually of sympathetic chain ('sympathectomy') were removed just above the site of passage of these structures through the diaphragm. The splanchnic nerve almost always presented itself as a single cord; greater and lesser subdivisions were usually not identified.

Except in the 'acute' experiments, renal clearances were usually measured at least two weeks after the last adrenal or splanchnic operation. Each ureter was approached retroperitoneally, severed, and a no. 6 French ureteral catheter securely tied in. The position of the catheter was adjusted carefully throughout the remainder of the experiment to prevent obstruction of urine flow due to angulation of the ureter.

*Technique of renal clearance measurement.* Renal clearances were measured with the animal anesthetized with nembutal, in the supine position. The infusion mixtures usually consisted of a) a 'priming' solution of 15 cc. of 25 per cent mannitol solution and 0.5 cc. of 20 per cent sodium para-aminohippurate ('PAH') solution and b) a 'sustaining' solution of 1.6 per cent mannitol and 0.12 per cent PAH in 0.9 per cent aqueous solution of NaCl. The latter solution was administered by continuous intravenous drip at a rate of 3 to 4 cc. per minute. Urine was collected directly from the ureteral catheters into small volumetric flasks. Clearance periods were usually 30 minutes long. An infusion of 100 to 300 cc. of 0.9 per cent saline solution was always given before starting the clearances in order to establish a good urine flow.

In some experiments it was thought desirable to increase the urine flow or the urinary chloride concentration in the later clearance periods. For these purposes, 100 cc. of 2 per cent or 5 per cent NaCl was administered in place of the usual sustaining mixture at a rate about double that employed for the sustaining mixture, and the concentrations of mannitol and PAH in the hypertonic solutions were accordingly reduced. Arterial blood samples were collected either from the femoral artery or from the heart at approximately the mid-points of the clearance periods. The aspirating syringe and the test tube receiving the blood each contained two drops of one per cent heparin solution. In later experiments (dog 14 and after) the blood was collected under oil and iced until centrifugation. Centrifugation of the blood and separation of the plasma were performed usually within an hour after bleeding.

In those clearance experiments in which epinephrine was administered, the animal received 10

to 14 gamma of the hydrochloride per minute. Where pituitary adrenocorticotrophic hormone<sup>3</sup> was administered, an aqueous solution containing 20 mg. was given intravenously over the course of one minute.

Post-mortem examination was performed in all animals in order to verify the presence or absence of adrenal tissue, the interruption of nerve tracts and the presence of grossly normal kidneys bilaterally.

The calculations of the renal clearances of mannitol and PAH were determined according to the method of Möller, McIntosh and Van Slyke (11), except that plasma rather than whole blood was used. Therefore, the term 'clearance' refers to plasma clearance.

*Analytical techniques.* Mannitol was measured by the periodate-thiosulfate titration technique of Smith *et al.* (12). Cadmium sulfate was used to precipitate protein from plasma and urine, as recommended by Goldring and Chasis (13). Among factors which were considered in performing the plasma and urine blanks were nonfermentable reducing substances contributed by the plasma and urine, or 'Factor 1', reducing substances contributed by the yeast suspension,<sup>4</sup> or 'Factor 2', and adsorption or destruction of mannitol by the yeast suspension, or 'Factor 3'. In performing the plasma blank during determinations of the mannitol clearance for dogs 1-7 Factors 1 and 2 were compensated for. For dogs 8-23 a blank was employed which compensated for only Factor 1. All three factors were taken into consideration for dogs 24-28. As regards the urine mannitol blank, no blank was applied until the experiment on dog 22 at which time there was introduced a blank compensating for Factors 2 and 3. A urine blank compensating for all three factors was employed only for dog 26. Details of the method employed for calculation of the blank will be presented in a separate report (14).

These variations in technique influenced the calculated values for the renal clearances to a significant degree. However, the effects of variation in the analytical methods, applying equally as they do to the observations made on both kidneys, are not likely to invalidate conclusions based on comparisons of the simultaneous functions of the right and left kidney.

Para-aminohippurate was measured as recommended by Smith *et al.* (15). The plasma blank was determined after addition of a known quantity of PAH to a protein-free filtrate. The urine PAH blank was found to be insignificant in one dog and was not corrected for in any experiment.

The chloride content of plasma was determined as described by Van Slyke (16); that of urine was measured by the Volhard-Arnold technique (17).

## RESULTS

In general, in this paper reference to an 'increase' in excretion of any urinary constituent refers to a greater output of that constituent on the side in question *as compared to the contralateral side*. Thus, the term 'increase' does not necessarily refer to an absolute increase. Data on a representative experiment from each of the groups described below are presented in table 1. Average values of excretory rate ratios for all the satisfactory experiments in the various groups are shown in table 2; data obtained following the injection of epinephrine or adrenocorticotrophic hormone are excluded from this table.

*Controls.* The results of studies in 6 control dogs are shown in table 2, the only operation performed being the insertion of the ureteral catheters. In these animals the ratio of the excretion rate of each of the substances for the left kidney to the rate for the right kidney usually approximated unity. Data on an additional control dog were excluded because of the presence of ureteral obstruction, since this factor

<sup>3</sup> Donated by Armour & Co., Chicago, Ill. This preparation, lot 37-KE, contained 0.0025 U.S.P. units of the oxytocic factor and 0.005 units of the pressor factor of the posterior pituitary gland per milligram.

<sup>4</sup> Starch-free baker's yeast donated by Anheuser-Busch, Inc., St. Louis, Mo.



TABLE 1. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON SIMULTANEOUS EXCRETORY RATES FOR EACH KIDNEY—REPRESENTATIVE EXPERIMENTS

ANIMAL	OPERATION	PER.	URINE				PLASMA CHLORIDE	PLASMA CLEARANCE				EXCRETORY RATE, LT. KIDNEY				COMMENT
			Volume		Chloride			Mannitol		PAH		EXCRETORY RATE, RT. KIDNEY				
			Rt.	Lt.	Rt.	Lt.		Rt.	Lt.	Rt.	Lt.	Mannitol	PAH	Water	Chloride	
			cc. per min.	cc. per min.	mEq. per l.	mEq. per l.		mEq. per l.	cc. per min.	cc. per min.	cc. per min.	cc. per min.				
Dog 6. 31 lbs.	Control	1	0.71	0.69	78.1	71.5	114.7	17.1	16.5	58.6	55.3	0.96	0.94	0.97	0.90	5% NaCl
		2	1.41	1.30	142	138	125.9	17.1	16.8	46.9	43.4	0.95	0.93	0.92	0.90	
		3	1.54	1.45	152	152	124.5	16.5	16.1	45.3	44.6	0.97	0.98	0.94	0.94	
Dog 3. 22 lbs. 17 days <sup>1</sup>	Lt. adrenalectomy, chronic	1	1.36	1.89	68.1	82.9	111.7	25.8	26.6	50.7	55.4	1.03	1.10	1.40	1.70	5% NaCl
		2	0.97	1.88	76.2	110	112.1	29.2	31.4	72.7	79.4	1.08	1.09	1.95	2.79	
		3	1.29	2.20	128	148	122.2	31.5	32.3	92.0	102	1.02	1.11	1.70	1.98	
		4	1.69	2.60	174	178	129.8	29.1	31.4	78.6	94.2	1.08	1.20	1.54	1.57	
		5	1.75	2.19	185	186	129.3	31.0	32.0	80.5	92.0	1.03	1.14	1.25	1.26	
		6	1.65	2.08	196	198						1.05	1.19	1.27	1.27	
Dog 15. 34 lbs.	Lt. adrenalectomy, acute	1	0.69	0.72	146	138	112.5	28.8	29.1	106	108	1.01	1.01	1.04	0.94	Lt. adrenalectomy; 64 min. elapsed between per. 2 & 3
		2	0.58	0.54	137	122	113.1	26.1	25.2	89.7	92.5	0.97	1.03	0.93	0.83	
		3	0.51	0.87	54.2	85.5	117.7	27.9	29.4	79.5	81.9	1.05	1.03	1.72	2.71	
		4	0.52	0.97	76.3	104	121.0	24.7	29.2	67.6	75.6	1.18	1.12	1.87	2.55	2% NaCl
		5	0.59	1.08	110	131	123.4	26.8	28.7	73.9	76.4	1.07	1.03	1.83	2.21	
		6	1.35	2.03	156	160	130.7	29.5	31.9	102	109	1.08	1.07	1.50	1.54	5% NaCl
Dog 8. 31 lbs. 13 days <sup>1</sup>	Lt. splanchnic resection; lt. sympathectomy, chronic	1	0.59	2.11	78.3	160	112.7	28.8	34.4			1.19		3.59	7.36	2% NaCl
		2	0.37	1.77	54.0	166	111.9	29.5	32.7			1.11		4.79	14.7	
		3	0.65	1.60	117	169	113.2	31.7	36.0			1.14		2.46	3.55	
		4	0.56	1.33	88.0	153	113.8	33.8	38.5			1.14		2.36	4.11	
		5	0.62	1.03	126	159	114.9	28.1	30.6			1.09		1.66	2.09	Epinephrine
Dog 13. 20 lbs.	Lt. splanchnic resection, acute	1	0.66	0.68	64.6	78.8	113.3	23.1	23.6	66.7	70.9	1.02	1.06	1.02	1.25	Lt. splanchnic resection; 46 min. elapsed between per. 2 & 3
		2	0.72	0.79	93.1	105	114.5	23.3	24.5	66.3	69.7	1.05	1.06	1.09	1.22	
		3	0.51	1.73	66.0	153	116.5	21.2	25.4	62.1	69.7	1.20	1.12	3.38	7.84	
		4	0.98	2.04	120	158	124.4	21.2	22.8	55.8	66.3	1.08	1.19	2.99	3.94	2% NaCl
		5	1.68	4.77	155	176	140.2	20.9	25.0	52.3	61.2	1.20	1.17	2.84	3.23	5% NaCl
		6	1.29	4.45	150	178	137.7	20.1	23.0	49.6	57.5	1.16	1.16	3.46	4.08	
Dog 26. 31 lbs. 25 days <sup>1</sup>	Bilateral splanchnic resection, bilateral sympathectomy, lt. adrenalectomy	1	1.91	1.74	143	142	111.0	33.5	32.3	107	101	0.97	0.95	0.91	0.90	ACTH <sup>2</sup> 20 mg. I.V. start of per. 4
		2	2.18	1.80	150	146	109.6	33.9	32.8	103	102	0.97	0.99	0.83	0.81	
		3	2.04	1.73	153	150	107.6	33.9	32.9	102	101	0.97	0.99	0.85	0.83	
		4	2.00	1.49	158	152	109.6	36.3	34.2	105	103	0.95	0.98	0.75	0.72	
		5	1.97	1.81	157	165	110.5	33.8	33.5	96.4	96.2	1.00	1.00	0.92	0.97	
		6	1.61	1.40	162	160	110.9	34.5	34.1	104	102	0.99	0.98	0.87	0.86	
		7	1.58	1.35	175	172	111.1	36.8	36.8	115	117	1.00	1.02	0.86	0.85	

<sup>1</sup> Time interval between operation and measurement of excretory rates.    <sup>2</sup> Pituitary adrenocorticotrophic hormone.

causes a diminished output of urine and chloride (19, 20) as well as a decreased rate of glomerular filtration (21).

*Unilateral adrenalectomy—chronic.* In 3 out of 4 dogs, removal of the adrenal gland resulted in a markedly greater excretion of water and chloride and a slightly but consistently greater excretion of mannitol and PAH by the homolateral kidney as compared with the control kidney (e.g., dog 3, table 1). In the mannitol excretion ratios of 2 animals there were intermittently observed deviations from unity which were not in excess of the deviations observed in the control animals (compare dogs 3 and 6, table 1); however, these variations in the adrenalectomized animals were accompanied by deviations in the excretion of water and chloride which were more striking than those in the control group. In the one animal (dog 22) responding atypically, no appreciable increase was demonstrated in any of the urinary constitu-

TABLE 2. INFLUENCE OF VARIOUS OPERATIVE PROCEDURES UPON AVERAGES OF EXCRETORY RATE RATIOS FOR VARIOUS SUBSTANCES

OPERATION	NO. ANIMALS	NO. PERIODS OF OBSERV.	EXCRETORY RATE, LT. KIDNEY EXCRETORY RATE, RT. KIDNEY			
			Mannitol	PAH	Water	Chloride
Control.....	6	14	1.00	1.00	1.02	1.06
Lt. adrenalectomy.....	5	22	1.06	1.08	1.67	2.20 <sup>1</sup>
Lt. splanchnic resection <sup>2</sup> .....	3	14	1.18	1.19	3.05	5.47
Bilateral splanchnic resection; left adrenalectomy <sup>3</sup> .....	4	11	1.01 <sup>1</sup>	1.01 <sup>1</sup>	0.97 <sup>1</sup>	0.99 <sup>1</sup>

<sup>1</sup> Statistical analysis by a method applicable to small samples (18) indicates that the differences between these values and the control values are not statistically significant ('t' less than 2.5).

<sup>2</sup> The left sympathetic chain was also sectioned in 2 animals in this group.

<sup>3</sup> The sympathetic chain was also sectioned bilaterally in 3 animals in this group.

ents; in the discussion below the explanation will be suggested that a minimum of damage was caused to the sympathetic nerves of this animal during adrenalectomy. Data on 3 other animals in this group were excluded from table 2 because of the presence of ureteral obstruction, severe emaciation, and chronic infection, respectively.

*Unilateral adrenalectomy—acute.* The effects of unilateral adrenalectomy were evaluated in one animal by comparing the clearances on the two sides immediately before and after excision of the gland (dog 15, table 1). The increased excretion of water and chloride on the homolateral side immediately following the operation was striking. As in the case of dog 3 (table 1) and dog 4 (unilateral adrenalectomy), these changes were accompanied by only a relatively small increase in mannitol clearance. A summary of data on the 3 animals which had undergone unilateral adrenalectomy is presented in table 2.

*Unilateral splanchnic nerve resection—chronic.* Since it was known that resection of the splanchnic nerves resembles adrenalectomy in that it also results in an increased output of chloride and water by the homolateral kidney in the anesthetized animal (2, 6, 22), it was desirable to compare the effects of supradiaphragmatic

splanchnic resection with those of adrenalectomy, especially with regard to the clearance of mannitol and PAH. In 2 dogs resection of the splanchnic nerve and the sympathetic chain resulted in a greater diuresis of chloride and water than was produced by adrenalectomy (e.g. dog 8, table 1).

*Unilateral splanchnic nerve resection—acute.* In a single acute experiment (dog 13, table 1) in which preliminary control clearances were performed, the markedly increased output of chloride and water immediately after splanchnic resection was striking; a moderate increase in PAH and mannitol excretion was also observed. These findings indicate that the acute and chronic effects of unilateral splanchnic resection are similar. Maintenance of the integrity of the sympathetic trunk in the acute experiment did not qualitatively modify the response noted in the chronic experiment (dog 8, table 1). The data on the 5 animals which underwent unilateral splanchnic resection are summarized in table 2.

*Bilateral splanchnic resection and unilateral adrenalectomy.* It soon became apparent that the results following unilateral adrenalectomy were similar to those following unilateral splanchnic resection. Three explanations for the effect of adrenalectomy therefore seemed possible: 1) unavoidable injury to nerves, 2) interruption of flow of adrenal hormones to the kidney or 3) a combination of these two mechanisms. In order to distinguish a primary adrenal influence from a nervous influence, a comparable nerve lesion was produced on both sides by a bilateral supra-diaphragmatic splanchnicectomy; in addition, unilateral left adrenalectomy was performed. After bilateral transection of the splanchnic nerves, any effect observed following unilateral adrenalectomy would probably be due to removal of adrenal hormones from the blood supply to the homolateral kidney. The results of experiments on 4 animals made it apparent that adrenalectomy did not cause a homolateral increase in excretion of chloride and water after splanchnicectomy (table 2, and dog 26, table 1). No consistent correlation between the mannitol excretion ratios and the urine or chloride excretion ratios was observed.

However, there still remained the possibility that the remaining intact, but denervated right adrenal gland in these animals was incapable of discharging epinephrine (25), a substance known to augment the output of adrenal corticosteroids (24, 25) by stimulating the anterior pituitary to produce adrenocorticotrophic hormone (26). Hence, this denervated adrenal may not have produced amounts of cortical hormone sufficient to influence homolateral renal function via the hypothetical direct adrenal-renal vascular channel. In order to ensure adequate stimulation of the remaining adrenal, pituitary adrenocorticotrophic hormone was administered intravenously to 2 of the 4 animals already referred to as having undergone bilateral sympathectomy and unilateral adrenalectomy. The results in these 2 animals (e.g. dog 26, table 1) indicated that adrenocorticotrophic hormone exerted no immediate differential effect upon the function of the two kidneys. Evidence of one aspect of the hormone's biological activity was provided in both animals by the observed increase in the ratio of the excretion of uric acid to that of creatinine, as described by Forsham *et al.* (27). Epinephrine was administered to the 2 other dogs, in part for the purpose of stimulating the adrenal cortex indirectly, via the pituitary (26); this drug also failed to exert a differential effect upon the function of the two kidneys.

TABLE 3. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON RELATIVE WATER AND CHLORIDE REABSORPTION RATES FOR EACH KIDNEY—REPRESENTATIVE EXPERIMENTS

ANIMAL	TYPE OPERATION	PERIOD	PLASMA CHLORIDE mEq./lt.	MANNITOL U/P		MANNITOL U/P <sup>1</sup> CHLORIDE U/P		COMMENT
				Rt.	Lt.	Rt.	Lt.	
Dog 6	Control	1	114.7	24.0	23.8	35.4	38.3	5% NaCl
		2	125.9	12.6	13.0	11.2	11.9	
		3	124.5	10.7	11.1	8.78	9.10	
Dog 3	Lt. adrenalectomy, chronic	1	111.7	19.0	14.1	31.1	19.1	5% NaCl
		2	112.1	30.2	16.7	44.4	17.1	
		3	122.2	24.4	14.7	23.2	12.1	
		4	129.8	17.2	12.1	12.8	8.84	
		5	129.3	17.0	14.6	11.9	10.2	
Dog 15	Lt. adrenalectomy, acute	1	112.5	41.9	40.4	32.5	33.1	Lt. adrenalectomy performed be- tween periods 2 & 3
		2	113.1	44.8	46.4	37.0	42.9	
		3	117.7	55.2	33.8	120.	46.6	
		4	121.0	47.6	29.9	75.7	34.7	
		5	123.4	45.1	26.6	50.4	24.8	
		6	130.7	21.9	15.7	18.4	12.8	
Dog 8	Lt. splanchnic re- section, chronic	1	112.7	49.1	16.3	70.9	11.5	2% NaCl  Epinephrine
		2	111.9	79.6	18.5	165	12.5	
		3	113.2	48.8	22.5	47.4	15.1	
		4	113.8	59.8	28.9	77.5	21.6	
		5	114.9	45.1	29.4	40.9	21.3	
Dog 13	Lt. splanchnic re- section, acute	1	113.3	35.0	34.9	61.4	50.2	Lt. splanchnic re- section per- formed between periods 2 & 3
		2	114.5	32.3	31.1	39.7	33.9	
		3	116.5	41.3	14.7	72.8	11.2	
		4	124.4	21.5	7.90	22.3	6.21	
		5	140.2	12.4	5.22	11.2	4.14	
		6	137.7	15.6	5.24	14.3	4.06	
Dog 19	Bilateral splanchnic resection, lt. ad- renalectomy	1	110.1	17.7	17.0	16.1	15.2	Epinephrine Epinephrine
		2	109.7	20.9	19.1	18.8	17.1	
		3	110.7	21.6	21.4	19.4	19.5	
		4	108.6	26.6	26.9	28.7	27.5	
		5	109.4	18.4	17.3	17.0	16.4	

<sup>1</sup> 'Chloride reabsorption ratio'.

*Other effects of epinephrine.* Epinephrine administered intravenously with the clearance solutions to dog 14 (left adrenalectomy) tended to equalize the mannitol and PAH clearances in the two kidneys and abolish the marked disproportion in chloride and water excretion. A quantitatively smaller, but similar effect was seen in dog 8 (left splanchnic resection, transection of sympathetic chain, table 1). However in 3 dogs (18, 19 and 22) in which there was no initial inequality in kidney function as regards the two sides despite left adrenalectomy with or without bilateral splanchnicectomy, epinephrine had little or no effect on the relative rates of excretion.

*Tubular reabsorption of chloride and water.* Our data permitted a calculation of the amount of chloride and water reabsorbed from the glomerular filtrate during each clearance period. On the assumption that mannitol is not absorbed from the tubules, we have employed the ratio  $\frac{\text{urine mannitol concentration}}{\text{plasma mannitol concentration}}$ , also designated

TABLE 4. EFFECT OF VARIOUS OPERATIVE PROCEDURES UPON THE AVERAGES OF RELATIVE WATER AND CHLORIDE REABSORPTION RATES FOR EACH KIDNEY

OPERATION	NO. ANIMALS	NO. PERIODS OF OB- SERV.	MANNITOL U/P		MANNITOL U/P CHLORIDE U/P	
			Rt.	Lt.	Rt.	Lt.
Control.....	6	14	24.0	23.8	28.2	27.4
Lt. adrenalectomy.....	5	20	26.6	17.4	36.9	20.2
Lt. splanchnic resection.....	3	15	35.2	14.9	53.9	12.0
Bilateral splanchnic resection; left ad- renalectomy.....	4	11	24.7	25.6	21.0	20.8

'Mannitol U/P', to represent the degree to which the glomerular filtrate is concentrated by the reabsorption of water.

Any substance which is partially reabsorbed (such as chloride, abbreviated 'Cl') is characterized by a U/P ratio less than the simultaneous mannitol U/P, the discrepancy being dependent on the amount reabsorbed. Hence, the  $\frac{\text{man U/P}}{\text{Cl U/P}}$  ratio

is always greater than 1.0; the higher the ratio  $\frac{\text{man U/P}}{\text{Cl U/P}}$  (hereinafter termed 'chloride

reabsorption ratio'), the greater the degree of reabsorption of chloride by the tubules. The values for this ratio determined simultaneously for the two kidneys in the same dog are shown in table 3; representative animals are chosen from experiments described above. The mannitol U/P ratios, indicative of the degree of water reabsorption, are shown for the same animals. In a control dog 6, and in the control periods of the acute experiments (dogs 13 and 15) both ratios on the two sides are nearly equal. They are also nearly equal in the bilaterally splanchnicectomized dog (dog 19) with one adrenal removed. In contrast, following either unilateral adrenalectomy or unilateral splanchnicectomy, the homolateral kidney invariably shows a lower chloride reabsorption ratio and mannitol U/P ratio than the control; this indicates a lesser degree of reabsorption of chloride and water in the homolateral kidney. Average values of the same ratios for all animals in the various groups studied are shown

in table 4; periods in which ureteral obstruction existed or drugs were administered are omitted.

#### DISCUSSION

*Explanation of increased excretion of water and chloride.* From the observed values for urine volume and chloride content, serum chloride and mannitol clearance, it may be calculated that in 3 dogs (period 3, *dog 3*, table 1); periods 2, 3 and 4, *dog 4* (chronic unilateral adrenalectomy); and periods 4, 5 and 6, *dog 13* (table 1) the small increments in volume of glomerular filtrate observed after unilateral adrenalectomy or splachnicectomy contributed water and/or chloride in amounts *less than* the extra amounts excreted. During all but two of these periods hypertonic solution of NaCl was administered. In the majority of periods of observation, the relative increment in glomerular filtrate observed to follow certain of the operative procedures provided amounts of chloride and water in excess of the extra amounts excreted by the homolateral kidney. However, this excess was not of great magnitude so that it was usually necessary to assume that an uncommonly large proportion of the 'extra' water and chloride filtered appeared in the urine with little reabsorption taking place in the tubules. That there is a relatively decreased tubular reabsorption of water and chloride following our operative procedures is also apparent from the decreased mannitol U/P and chloride reabsorption ratios presented in tables 3 and 4.

It is possible to interpret these findings as indicating that certain of our operative procedures had the specific effect of diminishing tubular reabsorption of chloride and water by direct action on the tubules. An alternative explanation is the possibility that the phenomenon is a consequence of the relatively increased glomerular filtration rate simultaneously observed. We have no information permitting a definite decision in this regard. In our own data we have noted no consistent relationship between small *spontaneous* changes in mannitol clearance in one kidney and the calculated chloride reabsorption ratio (table 3). However, in two experiments the relatively increased excretion of water and chloride in unilaterally adrenalectomized or splachnicectomized dogs was reduced by the injection of epinephrine (e.g. *dog 8*, tables 1 and 3). The fact that the mannitol clearance in the homolateral kidney fell concomitantly suggested that the filtration rate and the degree of reabsorption of water and chloride were related.

On the other hand, certain other observations suggest that our operative procedures may have altered renal function by means other than increasing glomerular filtration rate. It was apparent that in the *intact, anesthetized* dog, the excretion of water and chloride usually was approximately proportional to the filtration rate; i.e., if the rate of filtration in the right kidney exceeded that in the left kidney by 5 per cent, the excretion of chloride and water by the right kidney exceeded that by the left to very roughly the same degree. However, in the *unilaterally* adrenalectomized or splachnicectomized dog, the excretion of chloride and water was less closely proportional to the filtration rate; in these animals, an increase of 5 per cent in filtration rate in one kidney was occasionally accompanied by a relative increase in water and chloride excretion of 100 to 200 per cent (table 1).

The few observations on the effect of epinephrine in tending to equalize the

excretory activity of the two kidneys lend some support to the postulated specific influence of hormones from one adrenal gland upon the homolateral kidney. Unilateral adrenalectomy could produce its effects by abolishing the source of epinephrine, and unilateral splanchnicectomy could operate by interfering with liberation of epinephrine from the adrenal gland into the hypothetical adrenal-renal circulation. The fact that unilateral splanchnicectomy produces more striking changes in homolateral renal function than does adrenalectomy is evidence against the existence of a direct influence of secretions of the adrenal upon the homolateral kidney. Additional evidence was the absence of excretory changes in one animal (*dog 22*) following unilateral adrenalectomy.

*Mechanisms producing increase in renal blood flow and filtration rate.* Experimental interference with the transport of epinephrine to one kidney might be expected to result in vasodilatation in that organ and explain the observed increase in clearance of PAH and mannitol. It is more likely that any relative increase in renal blood flow in our experiments resulting either from adrenalectomy or splanchnicectomy was due to injury to the renal nerves rather than to interruption of any direct adrenal-renal vascular channel. The fact that the effects on the mannitol and PAH clearance following adrenalectomy were quantitatively less than those following splanchnic resection probably is an indication that adrenalectomy does not cause as extensive an interruption of nerve tracts leading to the kidney as does the latter operation. We interpret our failure to observe changes in excretion in *dog 22* (left adrenalectomy) as an indication that in this single instance we were able to remove the animal's left adrenal without injuring the renal vasomotor nerves. This experiment thus constitutes evidence that absence of one adrenal does not in itself influence homolateral excretion of chloride, water, mannitol, and PAH. Further proof is derived from the experiments on bilaterally splanchnicectomized animals; unilateral adrenalectomy performed on such animals did not result in a homolateral increase in renal clearances and chloride and water excretion even when the remaining intact adrenal was stimulated by adrenocorticotrophic hormone (table 1). Presumably, the adrenalectomy did not injure more renal vasomotor nerves than had already been sectioned during splanchnicectomy.

Analysis of our data affords no conclusion as to the anatomical location of the vascular readjustments which must necessarily have taken place to cause the increase in renal blood flow and glomerular filtration rate subsequent to unilateral adrenalectomy or splanchnicectomy. There was observed no consistent change in filtration fraction and, hence, definite evidence of changed glomerular arteriolar tone is lacking.

*Mannitol clearance as an index of glomerular filtration rate.* Recently some doubt has been expressed that the mannitol clearance is a true measure of the glomerular filtration rate (7) in the dog. The mannitol clearance in this animal is reported to be lower than either the simultaneous inulin or creatinine clearance. Other workers (28, 29) using dissimilar methods for the analysis of mannitol, reported that in human beings the ratio of the mannitol clearance to that of inulin approximates 0.9. If tubular reabsorption of mannitol does occur, all calculations of glomerular filtration rate dependent on the value of the mannitol clearance are clearly in error to some

extent. For instance, a calculation of filtered chloride might be falsely low by as much as 10 per cent. However, in the analysis of our experiments, any error in the calculated filtration rate in one kidney will be largely compensated for by an error of the same magnitude on the opposite side.

#### SUMMARY AND CONCLUSIONS

1) The simultaneous renal excretions of chloride, water, mannitol and para-aminohippurate have been determined for each kidney in anesthetized dogs under the following conditions: *a*) control, *b*) after unilateral adrenalectomy, *c*) after unilateral splanchnicectomy and *d*) after bilateral splanchnicectomy and unilateral adrenalectomy.

2) Unilateral adrenalectomy in the dog usually results in markedly greater excretion of chloride and water by the homolateral kidney as compared to the control kidney. The excretion of mannitol and para-aminohippurate is usually slightly greater on the homolateral side.

3) Unilateral splanchnic resection, with or without concomitant partial sympathectomy, produces changes which are qualitatively similar but quantitatively greater than those observed after unilateral adrenalectomy.

4) In the bilaterally splanchnicectomized dog, unilateral adrenalectomy fails to augment relatively the excretion of mannitol, para-aminohippurate, chloride, or water by the homolateral kidney, even when the remaining intact adrenal cortex is stimulated by the administration of adrenocorticotrophic hormone.

5) It is concluded that unilateral adrenalectomy specifically affected homolateral renal function only in so far as renal nerves were injured.

6) Following either unilateral adrenalectomy or unilateral splanchnic resection, the extra chloride and water excreted by the homolateral kidney occasionally cannot be accounted for by the accompanying slight increase in glomerular filtration rate. These two operative procedures both produce a relative decrease in the degree of tubular reabsorption of chloride. There is available insufficient evidence to permit a decision as to whether this decrease is due to a specific inhibition of tubular reabsorptive activity or to the increased rate of glomerular filtration.

The authors are indebted to Misses E. Houghton, A. Curtin, J. Finn, and to Mrs. D. Heady and Mrs. H. Weil for technical assistance in performing this study. Dr. Henry A. Schroeder made helpful suggestions for improving the manuscript.

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# ELECTROGRAM OF TURTLE HEART STRIP IMMERSSED IN A VOLUME CONDUCTOR

LEON CHURNEY, RICHARD ASHMAN AND EDWIN BYER

*From the Department of Physiology, Louisiana State University, School of Medicine*

NEW ORLEANS, LOUISIANA

THE electrical changes attending excitation and propagation can be understood only if the monophasic action potential curve is known.<sup>1</sup> The form of this curve may be determined experimentally and by mathematical analysis (1, 2). Theory indicates that the monophasic curve may be approximated by integrating the axial current curve (linear conductor); which, in turn, may be approximated by integrating the membrane current curve (volume conductor). The latter curve can be predicted semiquantitatively from potential theory (3, 4) and verified experimentally. By performing two successive integrations on this curve, one may determine the monophasic curve. If the relations postulated by the local circuit theory hold for heart muscle as well as for nerve, the calculated and the experimentally determined monophasic curves should be identical.

In demonstrating the interconvertibility of these curves we shall present a new graphical method for deriving the monophasic curve from the membrane current curve directly. Unfortunately, for reasons to be presented in the body of the paper, direct application of this method is not always practicable. However, since differentiation and integration are reciprocal operations, it follows that if we can select the monophasic curve which yields the experimentally derived membrane current curve, we shall have accomplished our purpose.

It is clear that such an analysis, no matter how successful, does not constitute a proof of our thesis, namely, that from the membrane current curve it is possible to determine the monophasic, and vice versa. It is necessary to demonstrate also that, when the form of the membrane current curve is drastically changed by controlled experimental procedures, the monophasic curve changes in the expected manner. This we shall do.

## MEMBRANE CURRENT CURVE

Linear ventricular and auricular strips were deeply immersed in Ringer's solution. Unipolar recording was employed in conjunction with a Cambridge All-Electric Electrocardiograph. Non-polarizable electrodes were used; the remote electrode being at least 12 cm. from the exploring electrode and presumably negligibly affected by current generated by the contracting muscle. Stimulation of one

Received for publication July 15, 1948.

<sup>1</sup>Throughout we speak of the monophasic action potential curve as if it were the graphic representation of the time course of depolarization and repolarization of the cell membranes. We are not unaware that we are measuring potential differences in the external circuit and that the experimental record is only an approximation of the monophasic as thus defined.

end of the strip was effected by a make and break shock in rapid sequence through silver electrodes from a Harvard inductorium. There was no evidence of polarization due to the stimulating current.

Under these experimental conditions it is possible, as has been stated, to predict the form of the curve on the basis of potential theory. Provided potential gradients exist between the active region of the muscle and the resting or less active regions lying ahead and behind, local currents will be generated by the resting regions (sources) and discharge into the active region (sinks). A wave of excitation passing longitudinally down the muscle strip will give rise first to a diphasic deflection (positive and negative) associated with depolarization. Then, after an isoelectric period, there will be a diphasic deflection in the reverse order (negative and positive) associated with repolarization. The volume conductor curve, then, is polyphasic (fig. 1, A and B).



Fig. 1. TIME COURSE OF MEMBRANE CURRENT. A. Ventricular strip. B. Auricular strip. Spontaneous beats.

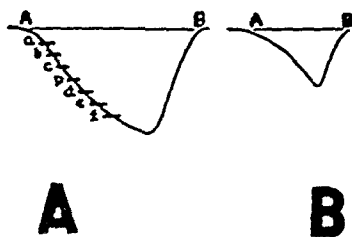


Fig. 2. CURVES ILLUSTRATING SPATIAL DISTRIBUTION of electrical density of auricular strip. A. Before applying mecholyl. B. After applying mecholyl.

The foregoing picture of excitation and recovery of heart muscle in a volume conductor has been given by Blair, Wedd and Young (5). Succinctly, their presentation involves two dipoles oppositely oriented and of equal strength passing under the recording electrode. But, as Macleod (6, 7) has pointed out, the repolarization process consists of a train of dipoles rather than just one. The distribution of the electrical density of this train in time, or space, is strictly related to the form of the descending limb of the monophasic curve. Our problem is to find a method for determining the intensities of polarization of the strip. Our solution is not explicit. Rather, it involves the representation of repolarization intensities by postulating a number of possible shapes for the descending limb of the monophasic curve. Graphical analysis of such curves yields experimentally verifiable membrane current curves.

Consider a strip of heart muscle,  $A-B$ , geometrically uniform and possessing homogeneous electrical properties in all its elements (fig. 2A). A wave of excitation is initiated at  $A$  and the potential changes are recorded at an intermediate point,  $p$ . (At  $p$  is a suction electrode which may be utilized to produce monophasic curves when desired.) Let the moment under consideration be the time when the wave of excitation has almost reached  $B$ . At this moment an observer at  $B$  looking backward sees

a contour for the total distribution of the electrical density of the strip from  $A$  to  $B$ . The membrane near  $B$ , not yet having been reached by the wave of excitation, bears the full complement of charges. Between  $f$  and  $B$  practically complete depolarization is assumed to exist. At  $c$ , however, recovery has begun and a few charges have been restored. Passing backwards to the point of stimulation,  $A$ , more and more charges have been restored. If the strip is long enough, 100 per cent repolarization will have taken place at  $A$ . We now make a fundamental assumption, namely, that the distribution of the charge density from  $f$  to  $A$  is given by the form of the descending limb of the monophasic curve recorded *in air*. Then the number of charges at  $c$ ,  $p$ , and  $d$  (unit areas equidistant from each other) is such that during repolarization  $p$  acts as a source relative to  $d$ , and as a sink relative to  $c$ . The overall effect at  $p$ , however, is that of a sink with respect to the remote electrode (*vide infra*). This presentation neglects effects from  $b$  and  $d$ ,  $a$  and  $f$ .

We may now plot the monophasic curve recorded in air at  $p$  on cross-section paper. The same curve is reproduced for point  $c$  to the left and, again, for  $d$  to the right of  $p$ . The horizontal distance between curves  $c$  and  $p$ ,  $p$  and  $d$ , is the assumed conduction time from  $c$  to  $p$ ,  $p$  to  $d$ , respectively. Since the wave of depolarization reaches  $c$  before  $p$  and  $p$  before  $d$ , the vertical distance at each instant between the ascending limbs of curves  $c$  and  $p$  is positive; that between  $p$  and  $d$ , negative. During repolarization, however, the vertical distances between the descending limbs of  $c$  and  $p$  become negative; those between  $p$  and  $d$ , positive. It is obvious, then, that the form of the membrane current curve can be approximated by *determining the difference in length at each instant of the verticals between curves  $c$  and  $p$ ,  $p$  and  $d$ , respectively*, taking care to interpret the sign correctly.

The foregoing procedure may perhaps be made clearer by using a diagrammatic representation (fig. 3). Assuming that the repolarization process is a monotonic function, two kinds of monophasic curves are recognizable on the basis of the form of the descending limb. In one the descending limb is concave downwards; in the other, concave upwards (fig. 3, B and C, respectively). A limiting intermediate form is illustrated in figure 3A, in which the descending limb is a straight line.

The simplest case is represented in figure 3A. Actually, descending limbs which are linear in form may appear transiently in the conversion experimentally of the curve of figure 3B to that of figure 3C. Polyphasic curves similar in appearance to the graphical resultant (fig. 3D) of figure 3A have been recorded. Such curves must not, however, always be conceived of as related to a monophasic curve whose descending slope is a straight line. An alternative interpretation will be given further on.

The monophasic curve illustrated in figure 3B has the usual configuration of heart muscle becoming repolarized under normal physiological circumstances, in that the descending limb is concave towards the time axis. The resultant membrane current curve (fig. 3E) has the following characteristics. The peak of the inward flowing current phase of the impulse is greater in magnitude than that of the outward flowing current density. The segment between the depolarization and repolarization deflections is everywhere slightly negative rather than isoelectric. This segment then dips into a trough whose maximum negativity is less, in absolute value, than the value for the crest of the succeeding wave of positivity. Assum-

ing that the descending limb of the monophasic curve is logarithmic (a very rough approximation for ventricular muscle), the time interval between the peaks of the diphasic deflection due to depolarization is equal to that between the peaks of the diphasic deflection due to repolarization.

Finally, the monophasic curve illustrated in figure 3C is the prototype of an experimentally recorded curve to be discussed further on. The essential feature of this curve is that it has the general form characteristic of nerve becoming repolarized under normal physiological circumstances, in that the descending limb is convex towards the time axis. The resultant membrane current curve (fig. 3F) is triphasic.

#### EFFECT OF LACK OF ELECTRICAL HOMOGENEITY OF THE MUSCLE

The experimental membrane current curves resemble in general figure 3E, which is the resultant of figure 3B; compare with figure 1, A and B. Variations do occur. Thus the maximum value for the inward flowing current phase of the impulse is not always just slightly greater than that for the outward flowing current density. In some instances it is very much greater in value; in others, much less. We are unable

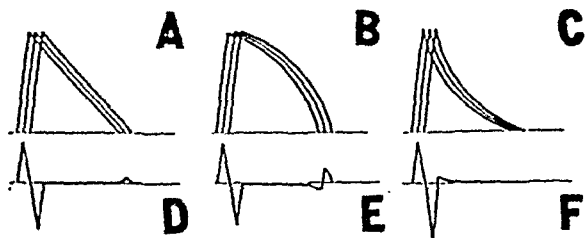


Fig. 3. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENTS, *D*, *E* and *F*, from the three types of curves, *A*, *B* and *C*, postulated for the monophasic action potential curve. See text. Ordinates in arbitrary units; those in *A*, *B* and *C* differing from those in *D*, *E* and *F*.

at present to explain this variability, though one or two obvious possibilities suggest themselves.

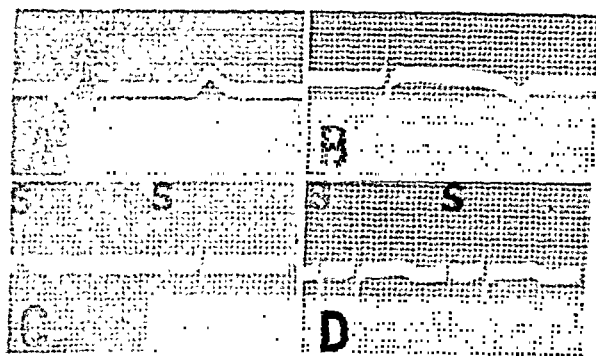
The segment connecting the depolarization and repolarization deflections is usually slightly below the isoelectric line, as predicted. Marked depression can be shown experimentally to be associated with effects due to nearby injured regions. Exaggerated elevation indicates injury under the electrode. By and large, our findings corroborate the studies of Eyster and Gilson (8).

More important for an understanding of conduction in heart muscle is a consideration of the time interval between the peaks of the diphasic depolarization deflection on the one hand and that between the peaks of the diphasic deflection due to repolarization. Our construction indicated that these time intervals should be equal, or nearly so. In our experimental curves the time interval between the peaks of the diphasic deflection of the impulse varies between one fifth to two thirds of the interval between the peaks of the diphasic deflection of the recovery process. The explanation for this discrepancy is that the tissue is not everywhere electrically homogeneous as was assumed in the theoretical treatment. It is well known that the elements of heart muscle show diverse rates of repolarization (9) and it is only because these rate differences are probably of a statistical character that the theoretically correct diphasic form is so frequently encountered experimentally.

We see, then, that the large preponderance of repolarization waves of the expected form is not only due to the sequence of depolarization of points *c*, *p*, and *d*

(fig. 2A), but is also contingent on the fact that near any point,  $p$ , picked at random, there will usually be areas of more rapid, and other areas of less rapid, repolarization. In several experiments we recorded not only from  $p$ , but also from a number of surrounding points. By this procedure, one can usually discover a point that yields a repolarization wave which is wholly positive (the region under the electrode becoming repolarized more quickly than, and therefore acting as a source for, closely adjacent regions) and another point that yields a purely negative repolarization wave (the region under the electrode becoming repolarized more slowly and behaving as a sink). In some records the repolarization wave is isoelectric, or almost so. This suggests that, provided the conduction velocity is constant (a valid assumption implicit throughout), the tissue under the exploring electrode,  $p$ , becomes repolarized just so much faster than that at  $c$ , but slower than that at  $d$ , that the effects cancel out. Finally, some records show a diphasic repolarization wave whose phases are reversed; i.e., in which the first phase is positive, the second negative. This may be

Fig. 4. ELECTROGRAMS ILLUSTRATING VARIATIONS in repolarization because of electrical non-homogeneity of the tissue. Compare with figure 1. A and B. Ventricular strips. C and D. Auricular strips. S, stimulus artifact.



explained by assuming that initially the region under the electrode acts as a local anodal focus, but is soon dominated by a more rapidly repolarizing adjacent region. Experimental records illustrating these four types of repolarization waves are given in figure 4. It is, of course, possible to construct graphically corresponding membrane current curves by changing the duration of the properly chosen monophasic curves,  $c$ ,  $p$ , or  $d$ , as each case demands.

That differences in the time course of repolarization of closely adjacent areas exist, and are responsible for the variations in the form of the deflections of the recovery process, may be more completely demonstrated by increasing the rate of stimulation and thereby reducing the cycle length. One of us has shown that this procedure effectively minimizes the differences in the time course of repolarization of the different muscle elements (10). A purely upright repolarization wave becomes diphasic as the cycle length is decreased and in figure 5 the diphasic character of this wave is seen to be accentuated. Furthermore, the interval between the peak of the negative phase and that of the positive phase of the repolarization wave is decreased. However, it is only when the time interval between the peaks of the diphasic depolarization deflection is unusually long (0.06 sec., instead of the more commonly recorded 0.01 to 0.03 sec. range) that this interval approaches that between the peaks of the diphasic repolarization deflection. We interpret this to mean that a residuum of electrical non-homogeneity is still present.

# EFFECT ON THE MONOPHASIC CURVE OF EXPERIMENTALLY MODIFYING THE MEMBRANE CURRENT CURVE

In figures 6A1 and 6B1 are shown typical monophasic records for ventricle and auricle respectively, recorded in air using the suction electrode. Below each experimental curve are shown: first, the diphasic axial current curves computed by numer-

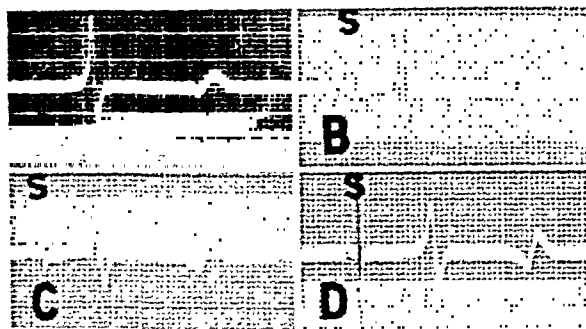


Fig. 5. ELECTROGRAMS ILLUSTRATING EFFECT of change of cycle length on repolarization. Cycle lengths: A. 8.6 sec.; B. 2.9 sec.; C. 2.1 sec.; D. 1.9 sec. Ventricular strip. S, stimulus artifact.

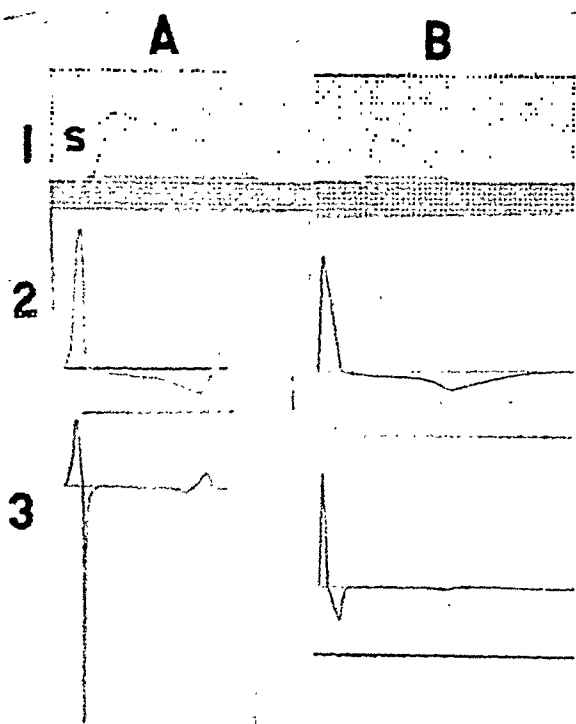


Fig. 6. A1 AND B1. MONOPHASIC ACTION POTENTIALS of ventricular and auricular strips, respectively, recorded in air with the suction electrode. Both show slight volume conductor effects. S, stimulus artifact. A2 and B2. Time course of axial currents of ventricular and auricular strips, respectively, derived by numerical differentiation. A3 and B3. Time course of membrane currents of ventricular and auricular strips, respectively, derived by numerical differentiation. Ordinates in arbitrary units and not comparable for different frames.

ical differentiation (figs. 6A2 and 6B2); and, secondly, the membrane current curves computed by numerical differentiation (figs. 6A3 and 6B3).

Copies of figures 6A1 and 6B1 are aligned for graphical analysis by our method in figures 7A1 and 7B1, respectively. The resultants are shown in figures 7A2 and 7B2.

It is instructive to compare the membrane current curves of figures 6A3 and 6B3, obtained by numerical differentiation, with those of figures 7A2 and 7B2 obtained by our graphical method and to compare both sets with the experimental records of figure 1. One sees that the resultants obtained by our graphical method

may provide a better basis for experimental prediction than those obtained by numerical differentiation. As a matter of fact, Cole and Curtis (11) have already pointed out the likelihood of lack of satisfactory prediction because of cumulative errors in the numerical calculation of the second derivative (and other reasons as well). As an aside, it may be of interest to note that our graphical method, when applied to the monophasic curve of Nitella, yields a maximum value of the inward current density which is definitely greater than that of the outward current density—a finding predicted by theory but not established by numerical differentiation. In general, it is quite clear that the relationships between the monophasic, di-, and polyphasic curves

Fig. 7. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENTS,  $A_2$  and  $B_2$ , from the monophasic action potentials,  $A_1$  and  $B_1$ .  $A_1$  and  $B_1$  are copies of the records of figure 6,  $A_1$  and  $B_1$ . Ordinates in arbitrary units and not comparable for different frames.

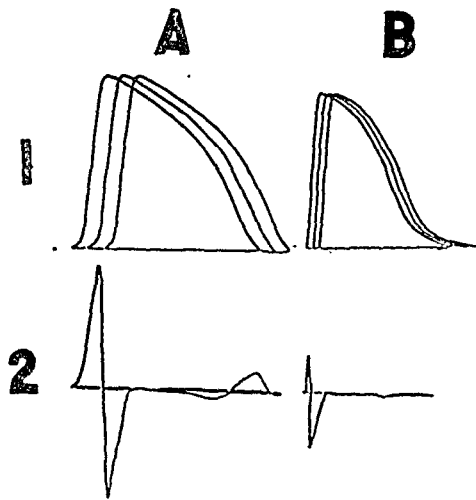
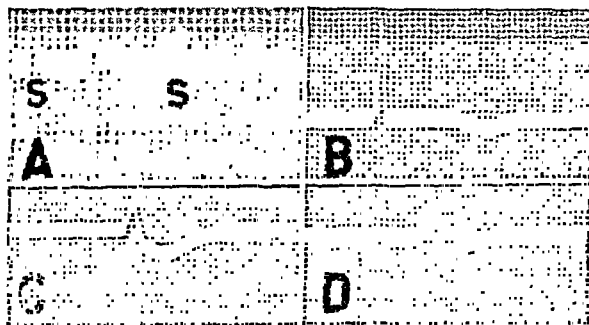


Fig. 8. A. TIME COURSE OF MEMBRANE CURRENT of auricular strip following application of mecholyl. B and C. Time course of axial currents of auricular strip before and after, respectively, applying mecholyl. D. Monophasic action potential of auricular strip following application of mecholyl. (Lead wires reversed from C.) S, stimulus artifact.



hold, so that the local circuit theory is valid for heart muscle under normal physiological conditions.

The validity of the theory would be greatly enhanced if it could be shown that the relationships hold under experimentally modified conditions. Accordingly we carried out some experiments on the effect of mecholyl on auricular strips immersed in a volume conductor. The effect of dilute solutions (c.  $10^{-5}$ ) of mecholyl is to convert the polyphasic form of the membrane current curve to a triphasic one like the resultant in figure 3F. The experimental record is shown in figure 8A.

Now, if our thesis is correct, namely, that, given a membrane current curve, it is possible to derive the appropriate monophasic curve, we should be able to record experimentally a monophasic curve for auricle treated with mecholyl which has the form shown in figure 3C. Linear strips in air were used. The normal diphasic action



potential curve was first recorded (fig. 8B; compare with fig. 6B<sub>2</sub>) and then mecholyl was placed on the tissue around one electrode. The diphasic curve underwent a series of changes, an example of which may be seen in figure 8C, until complete block supervened. The monophasic record is shown in figure 8D. Reoriented copies of the latter curve are arranged for graphical analysis in figure 9A. Obviously, experiment and theory are in good agreement. The graphical resultant is given in figure 9B; compare with figures 8A and 10B<sub>3</sub>.

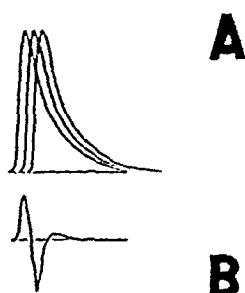


Fig. 9. GRAPHICAL CONSTRUCTION OF TIME COURSE OF MEMBRANE CURRENT, *B*, from the monophasic action potential, *A*. *A* is a copy of the record of figure 8D. Compare figure 9B with figure 8A. Ordinates in arbitrary units, those in *A* differing from those in *B*.

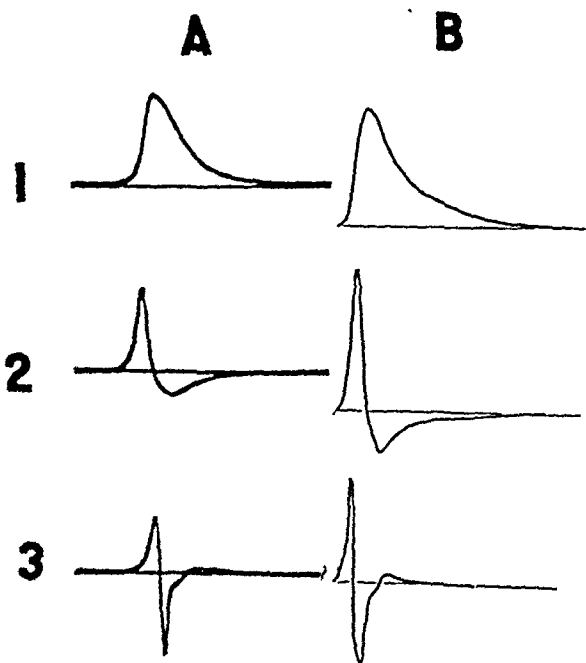


Fig. 10. COMPARISON OF TIME COURSE of monophasic action potentials, axial, and membrane currents of crab nerve (A<sub>1</sub>, 2, and 3, respectively) and of turtle auricular strip after applying mecholyl (B<sub>1</sub>, 2, and 3, respectively). Figures 10A<sub>1</sub>, 2, and 3 from figure 5 of Katz and Schmitt (18). Note that the time required for the inscription of the monophasic action potential of crab nerve is 4 msec.; of the turtle auricular strip, nearly 0.8 sec. Ordinates in arbitrary units and not comparable for different frames.

The profound change in the monophasic curve under the influence of mecholyl implies a modified distribution of the electrical charges on the auricular strip. We may now picture the contour for the distribution of the electrical density as in figure 2B. The principal change is the great decrease in length of the almost completely depolarized segment. This, we feel, is mainly due to an acceleration of the repolarization process. If the acceleration is sufficiently great, two effects may be expected. In the first place, currents derived from recovering or recovered regions will interfere at the active segment with currents originating from resting regions in front of the excitation wave. As a result, currents flowing out of the resting regions ahead of the impulse will meet with considerable resistance and be diminished in magnitude. Experimental evidence for this point of view is seen in the fact that for the auricular

strip treated with mecholyl the outward current density is very definitely less than that of the inward current density (fig. 8A). We venture to suggest that in nerve, normally, the repolarization dipole is advanced spatially, or temporally, so that its negative charge summates with that of the depolarization dipole. The magnitude of the positive charge of the depolarization dipole, as registered by the exploring electrode which it is approaching, will appear effectively lessened in magnitude.

The more obvious effect of the acceleration of recovery will be, as already stated, to decrease the length of the active segment. In terms of the dipole theory, this implies that the distance between the negative and positive charges of the repolarization dipole is decreased. One should bear in mind that in heart muscle the repolarization process is more accurately represented by postulating that the components of the repolarization dipole are more widely separated than those of the depolarization dipole (3).

Another, and equivalent, picture may be derived from an analogy with the reactions of ephapses (12, 13). When active and resting nerve fibers are contiguous over a sufficient length, the impulse generates currents which are, successively, anodal, cathodal, and anodal, in their effects on the resting fiber. Actually, the cathodal phase is double and appears as such in the normal heart muscle strip. The effect of mecholyl is to telescope these separate cathodal phases into one. The result is a picture like that of nerve (*vide infra*). Also, Arvanitaki (14) has shown for such double nerve preparations that in many of the geometrical arrangements the terminal anodal effect suppresses the active response of the resting fiber. In the case of the auricular strip treated with mecholyl, the activity of the depolarizing cathode may be thought of as if it were being aborted by the action of the terminal anode.

The foregoing presentation provides a point of departure from the conduction theories of Rashevsky (15) and Rushton (16). Their analyses postulate that the excitatory disturbance in effect leaves the tissue (nerve) permanently altered behind it. Electrical changes involved in recovery are assumed not to affect the electrical phenomena occurring in regions lying ahead of the excitation wave. In this connection see the paper of Offner, Weinberg and Young (17).

Having demonstrated that the local circuit theory of conduction in heart muscle is valid, and that our method of analysis of the electrical changes is self-consistent and capable of extension and prediction, we may be said to have accomplished the task set for ourselves. Before concluding, however, we should like to point out the interesting resemblance between the records of the electrical changes in auricular muscle, following the application of mecholyl, and those in normal nerve.

In figures 10A1, 2, and 3, we reproduce from the paper of Katz and Schmitt (18; page 478, fig. 5) the monophasic, diphasic, and membrane current curves, respectively, of crab nerve. The monophasic curve is obtained by electric integration of the diphasic; the membrane current curve by electric differentiation of the latter. In figures 10B1, 2, and 3, we present the monophasic, diphasic, and membrane current curves, respectively, of auricular muscle treated with mecholyl. The diphasic and membrane current curves are derived by successive numerical differentiation of the monophasic curve. Although the absolute temporal relationships between the two sets of curves are, of course, of an entirely different order of magnitude, the relative temporal relationships are strikingly similar.

## SUMMARY

A new graphical method has been presented for deriving monophasic action potential curves from membrane current curves (volume conductor), and vice versa. The applicability of the method was tested by experimentally modifying the form of the membrane current curve and predicting the form of the monophasic curve. The prediction was verified by experiment. We conclude from this analysis that conduction in these heart muscle strips may be interpreted as though the tissue were a single fiber.

Variations that occur in the repolarization process are interpreted to mean that the tissue is not homogeneous in its electrical properties. Unless this phenomenon is absent, or recognized and adequately dealt with, the application of numerical methods of integration for deriving monophasic curves from membrane current curves may yield incorrect results. The difficulties can be resolved by assuming various forms of monophasic curves which, by trial and error, will give a fit between the graphical resultant and the experimental record.

Finally, from empirical considerations, we feel that any complete and adequate theory of conduction must take into account the effects of electrical changes in the recovery process upon those occurring ahead of the wave of excitation. Present theories fail to do this.

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# EFFECTS OF CHANGES IN POSITION OF THE HEART OF THE CHICKEN ON THE ELECTROCARDIOGRAM<sup>1, 2</sup>

PAUL D. STURKIE

*From the New Jersey Agricultural Experiment Station, Rutgers University*

NEW BRUNSWICK, NEW JERSEY

THE effects of changes in the position of mammalian hearts on the electrocardiograms have been reported by a number of workers. This paper, to the writer's knowledge, represents the first report on the effects of position changes of the chicken heart upon the electrocardiogram.

## METHODS

Before the heart was exposed, the electrocardiogram was taken with the bird lying on its back and anesthetized with pentobarbital sodium. The muscle from the sternum was then removed and the sternum and sternal ribs were cut away, leaving the coracoids and clavicle intact. As soon as the heart was exposed, its position was charted in relation to the limb leads. Needle electrodes were inserted in the muscles at the base of the wings and in the muscle of the left thigh. The relationship of the Lead lines to the long axis of the body of most birds was such that the apex of the heart had to be rotated from  $80^{\circ}$  to  $90^{\circ}$  to the left of the midline and approximately  $60^{\circ}$  to the right of the midline in order to be perpendicular to Lead lines III and II, respectively.

The heart was rotated only on its antero-posterior axis and with the pericardium intact. The pericardium was freed of its attachments at the apical end and a piece of thread was tied to it. Thus the heart was rotated by rotating the pericardial sac. Attempts at rotating the heart with the pericardium removed usually resulted in injury currents and this method was abandoned.

The left ventricle of the chicken heart is about three times as large as the right ventricle (I) and the apex is curved slightly to the right. In rotating the apex to the left, the heart tends to rotate on its longitudinal axis more than when rotated to the right. This is discussed more fully later.

The electrocardiograms were recorded with a direct-writing, moving-coil galvanometer (Cardiotron) manufactured by the Electro-Physical Laboratories, New York City. The records of the three Leads were taken consecutively on the same instrument.

In calculating the electrical axes, the records were magnified and the amplitude recorded in 0.25 mm. In all cases the algebraic summation of amplitudes I and III equalled or approached closely those of II. In most cases the variation was not more than 0.5 mm.

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Received for publication June 30, 1948.

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Laboratory of Avian Physiology.

<sup>2</sup> This work was made possible by a grant from the Borden Company.

## RESULTS

*Position of the Exposed Heart.* The position of the heart of the chicken, as Lewis (1) has shown, is almost vertical. The hearts of the 7 birds studied were in the midline or slightly to the left side with the apex slightly to the right. In 2 of the birds the apex formed an angle of  $+100^\circ$  and in the remainder the angles were from  $+95$  to  $+90^\circ$ .

*Normal ECG of the Chicken.* Lewis (1), working with 2 chickens, described the electrocardiogram in that species. His description, except for Lead I, is the same as the writer's.

The ECG of Lead I of the normal bird is the most variable of the limb leads. Based upon serial ECG's of over 70 adult chickens (2), it was found that the ECG's of Lead I are of two general types with respect to configuration. Of the birds studied about 63 per cent exhibited one of the types and, in a few cases, the same bird exhibited both types in serial ECG's. A description of the two types follows:

*Type A* is characterized by an upright *P*, a very small, abortive upright *R*, followed usually by a relatively prominent *S* wave, and usually an upright *T* wave; the *T* in some cases, however, may be flat or isoelectric.

*Type B* is characterized by an upright *P*, followed by a relatively prominent upright *R*, and no *S*, or a small *S* wave. The *T* wave may be isoelectric, slightly positive or slightly negative. There are variations of these types, but in most cases *R* is more prominent than the *S*, or the reverse.

In Leads II and III, the *R* wave is very small or absent, while the *S* wave is prominent. The *T* wave is positive. *P* III usually is upright.

*Changes in ECG Following Exposure of the Heart.* One of the most prominent changes noted in the ECG of the exposed heart, as compared to that of the unexposed heart, was the tremendous increase in amplitude in all leads following exposure. The significance of the amplitude changes will be discussed in another report. The configuration of the ECG in Leads II and III was changed very little after exposure, except that in Lead III the *P* wave became inverted in most cases. Before exposure of the heart, the *P* wave was upright in 5 of the 7 birds. At that time, 5 of the birds exhibited a *Type B* Lead I, and 2 *Type A*. After exposure of the heart, 6 of the birds showed *Type B*. There were no other significant changes in contours of the ECG's except that usually the *R* or *S* was relatively more prominent than before the heart was exposed.

*Effects of Rotation to the Left on RS and T.* The *S* wave is normally the main ventricular deflection in Leads II and III and also in Lead I in some cases. In 5 of the birds studied, the main ventricular wave in I was an *R* (fig. 1). In determining the changes in the amplitude and electrical axes of ventricular depolarization, *RS* has been used as the most reliable indicator of that change. In all cases *RS* I and III before and after rotation equalled or closely approached *RS* II. Most of the hearts were rotated  $45^\circ$  and  $80^\circ$  to the left and some were also rotated  $30^\circ$  and  $60^\circ$ . A summary of the results of all degrees of rotation on *RS* and *T* is shown in table 1.

*RS.* In 8 out of 12 observations of Lead I, following rotation, the *RS*'s were less positive or more negative, as indicated in table 1. Two *RS*'s were slightly more

positive, and one less negative after rotation. In one instance where the *RS* was positive, it showed no change after rotation.

In those birds where the main ventricular waves were upright in Lead I before rotation, they became, in most cases, negative after rotation; and the degree of

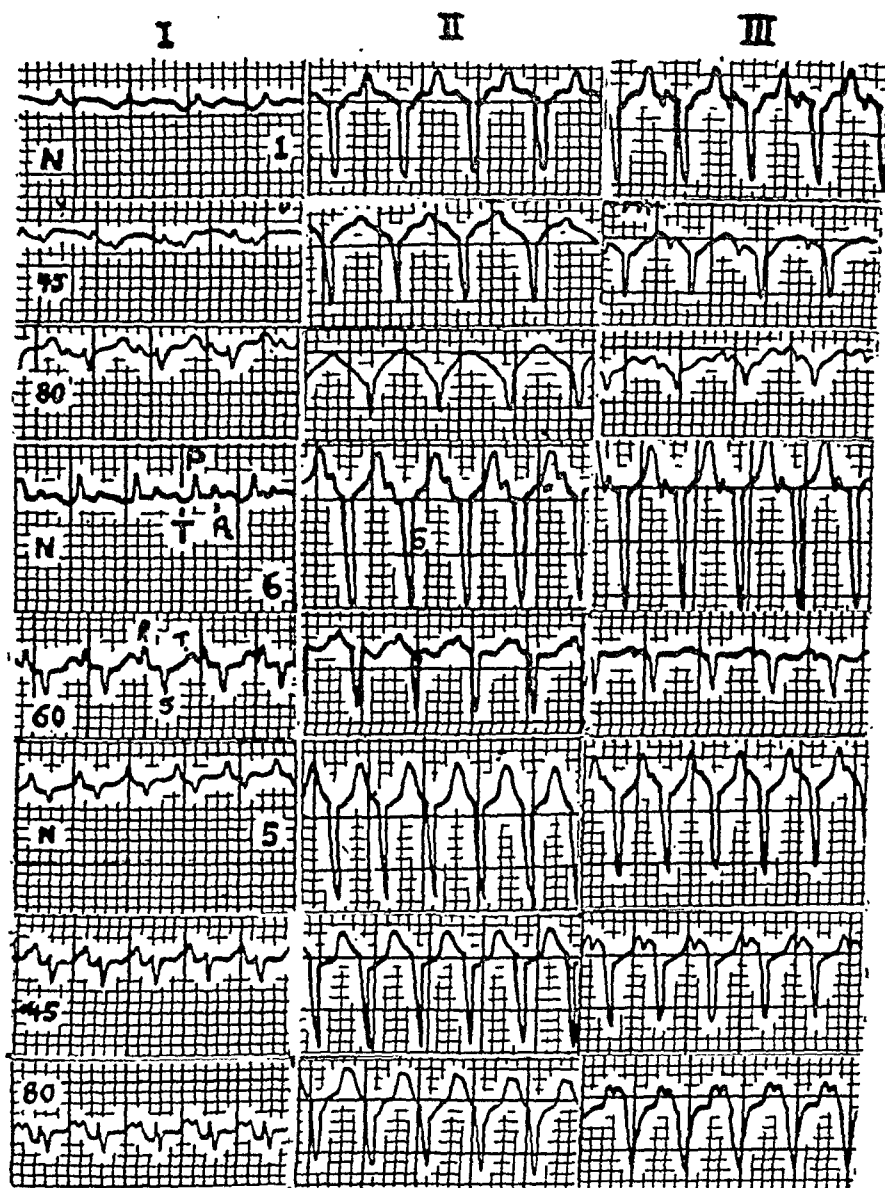


Fig. 1. ROTATION OF THE HEART on its antero-posterior axis to the left. Leads I, II, and III for birds 1, 5, and 6 before rotation (N) and after different degrees of rotation. Standardization, 1 mv.

negativity, in most instances, increased with the degree of rotation (fig. 1, birds 1 and 6). In two cases, the main ventricular waves before rotation were negative; and the *R*'s were absent or very small. After rotation, *R* waves appeared or increased, and the *S* waves decreased (fig. 1, bird 5).

In Lead II, 9 of the 13 cases exhibited a decrease in negativity in *RS* and 4 showed an increase. In 3 of these the change was slight and probably not significant whereas one showed a significant change. In 10 of 13 cases for Lead III, the *RS*'s

exhibited a decrease in negativity, 2 showed a slight increase (probably not significant), and one showed no change. The variation in degree of change of *RS* in Leads II and III varied considerably; however, the degree of change in III was in most cases greater than in II, particularly with the higher degrees of rotation (fig. 1).

Changes in electrical axes more clearly reflect the changes occurring in *RS* following rotation. The summary in table 2 shows that 11 of the *RS* axes were increased, and one was unchanged after rotation. The mean change in axes for all degrees of rotation was  $11.7^\circ$ , with a range of  $0^\circ$  to  $32^\circ$ . The maximum change was observed in bird 4. The axis for this bird before rotation to left was  $-68^\circ$ , which was appreciably lower than the axes for the other birds.

The hearts of 5 of the birds were rotated  $45^\circ$  and  $80^\circ$  or  $60^\circ$  and  $80^\circ$  to the left. In all instances, except one, the changes in electrical axes were greater with the higher degree of rotation, but the degree of change was not directly proportional to the degree of rotation. The degree of change with  $45^\circ$  rotation, in most instances, and with

TABLE 1. EFFECTS OF ROTATION OF HEART TO THE LEFT ON AMPLITUDE AND DIRECTION OF *RS* AND *T*.  
INCLUDES ALL DEGREES OF ROTATION

LEAD	NO. OF BIRDS	NO. OF ECG'S	MORE NEG. OR LESS POS.			LESS NEG. OR MORE POS.					NO CHANGE
			- to -	+ to -	+ to +	- to 0	- to +	- to -	0 to +	+ to +	
Change in RS											
I	6	12	1	5	2	1	0	0	0	2	1
II	7	13	4	0	0	0	0	9	0	0	0
III	7	13	2	0	0	0	0	10	0	0	1
Change in T											
I	6	12	0	0	0	0	4	2	2	3	1
II	7	13	0	0	13	0	0	0	0	0	0
III	7	13	0	0	13	0	0	0	0	0	0

<sup>1</sup> Sign of *RS* before rotation of heart. <sup>2</sup> Sign of *RS* after rotation of heart.

higher degrees of rotation in some instances, was of low order and probably not significant.

*T*. In Lead I, 11 of the *T* waves were less negative or more positive after rotation, and one showed no change (table 1). The *T*'s which were negative before rotation usually decreased in negativity or became positive after rotation and the degree of change increased with the degree of rotation (fig. 1). In Leads II and III, the *T* wave in all of the observations showed a decrease in positivity, and the degree of change was greater in III, in most cases.

The electrical axes for all of the *T* waves showed a decrease after rotation to the left. The mean change in axes was  $+29^\circ$ , with a range of  $+2$  to  $+77^\circ$  (table 2).

The more extensive change resulted from the greater degree of rotation, but the change was not proportional to the actual degree of rotation.

*Effects of Rotation to the Right on RS and T. RS.* The effects of rotation to the right on *RS* and *T* were more consistent and pronounced than the effects of rotation to the left. A summary of the results is shown in table 3.

In Lead I, eight of the nine *RS*'s which were positive before rotation (*R* more prominent than *S*, or *S* absent) increased in that direction after rotation (fig. 2, bird 7). The degree of change was pronounced in most instances. Of the four *RS*'s which were negative before rotation three of these were only slightly so (*R* almost as prominent as *S*) and they decreased or became positive after rotation. Only one bird exhibited a prominent *S* wave before rotation and this became positive after rotation. In Lead II, 12 *RS*'s before rotation were negative and all of these decreased in amplitude after rotation. One *RS* which was positive before rotation

TABLE 2. INCREASE (I) OR DECREASE (D) IN ELECTRICAL AXES FOLLOWING DIFFERENT DEGREES OF ROTATION

BIRD NO.	AXES BEFORE ROTATION		DEGREE OF ROTATION LEFT				DEGREE OF ROTATION RIGHT					
	RS	T	45°		80°		45°		60°		80°	
			RS	T	RS	T	RS	T	RS	T	RS	T
1	-89°	+87	-5I	+2D	-23I	+17D	-32D	+25I	-45D	+75I		
2	-80	+96	-8I	+2I	-6I	+19D			-12D	+9I	-17D	+27I
3	-114	+109	-6I	+48D								
4	-68	+78	-32I	+43D			-14D <sup>x</sup>	+78I				
5	-96	+85	-2I	+14D	-6I	+77D	-7D	+4I	-12D	+7I		
6	-85	+95	-27I <sup>1</sup>	+36D <sup>1</sup>	-19I	+50D			-4D	+13I	-13D	+20I
7	-80	+95	0	+9D	-7I	+30D	-9D	+8I	-28D	+42I	-28D	+44I

Summary: *RS*—11 increased, 1 no change; 12 decreased in angle; 12 increased in angle. *T*—12 decreased.

<sup>1</sup> 60° rotation. <sup>x</sup> = angle before rotation right (-16°). The axes of the other birds changed very little from those given.

TABLE 3. EFFECTS OF ROTATION OF HEART TO RIGHT ON AMPLITUDE AND DIRECTIONS OF *RS* AND *T* INCLUDES ALL DEGREES OF ROTATION

LEAD	NO. OF BIRDS	NO. OF ECG'S	RS MORE POS. OR LESS NEG.				NO CHANGE	T LESS POS. OR MORE NEG.				NO CHANGE
			- to 0	- to -	- to +	+ to +		- to -	+ to 0	+ to -	+ to +	
I	6	13	2	1	1	8	1	10	2	1	0	0
II	6	13	0	12	0	1	0	0	0	2	11	0
III	6	13	0	13	0	0	0	0	0	0	10	3

increased slightly after rotation. The electrical axes for all of the *RS*'s decreased after rotation (table 2). The average decrease was -18.4°, with a range of -4° to -45°.

*T. Changes.* In Lead I, all of the *T*'s became less positive or more negative after rotation of the heart to the right (table 3). The *T* wave normally may be slightly negative or positive. Ten of the *T*'s which were negative before rotation became more negative after rotation (fig. 2). Of the three cases which were slightly positive before rotation, two became isoelectric, and one became negative after rotation.

In Lead II, all of the *T* waves were upright before rotation, and 11 showed a decrease in positivity after rotation (fig. 2). In two cases the decrease was from



positive to negative. In Lead III, 10 of the  $T$ 's decreased in positivity and three showed no change after rotation. The degree of change in II was, in all instances, greater than in III.

The electrical axes of all the  $T$  waves increased after rotation. The average increase for all degrees of rotation was  $+20.3^\circ$  with a range of  $4^\circ$  to  $78^\circ$ . The degree of change in electrical axis for any given bird was greater with the higher degree of rotation, but there was considerable variation in the change between hearts receiving the same degree of rotation. The degree of change, however, was not directly proportional to degree of rotation. In most cases the actual change was less than the degree of rotation. In two exceptional cases (*birds 1 and 4*) where the hearts were rotated  $60^\circ$  and  $45^\circ$ , the changes in axes were  $75^\circ$  and  $78^\circ$  respectively.

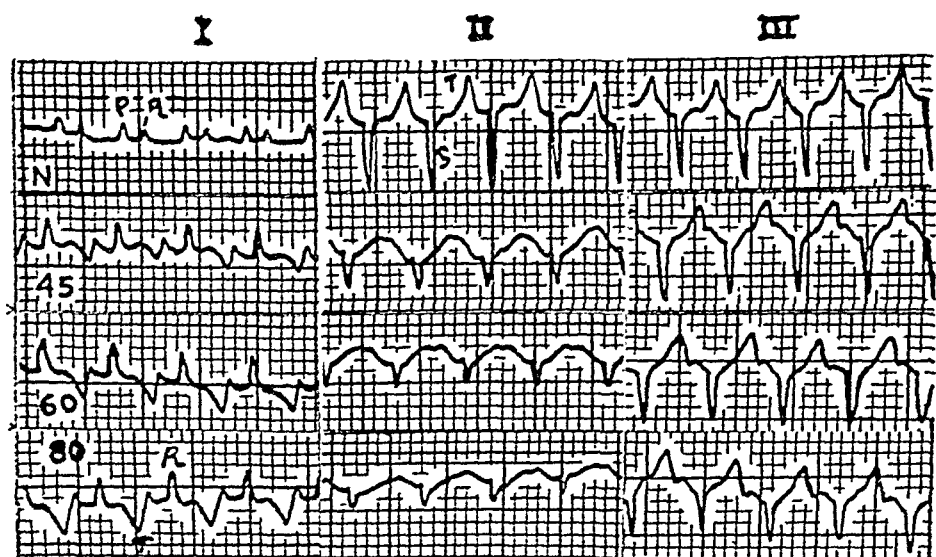


Fig. 2. ROTATION OF THE HEART on its antero-posterior axis to the right. Leads I, II, and III before rotation (N) and after different degrees of rotation. Standardization, 1 mv.

#### DISCUSSION

The results obtained in this study are fairly consistent with expectations based upon Einthoven's theory. In general, rotation of the heart to the left resulted in a great decrease in amplitude of  $S_3$  and  $T_3$ . The results obtained in Lead I depend upon the direction and configuration of  $R$ ,  $S$ , and  $T$  before rotation. In those cases where, in Lead I,  $R$  was relatively prominent (or  $S$  was absent) and the  $T$  wave was negative, rotation to the left resulted in a decrease in  $R$  and the appearance, or an increase of  $S$  and a positive  $T$  wave. When a relatively prominent  $S$  and a small upright  $T$  were present in I, before rotation, the  $S$  decreased and  $R$  and  $T$  increased after rotation. One of the birds studied was of this type. In this case, the decrease in amplitude of  $S_3$  was not so great as in the cases where an  $R$  wave was present in Lead I, as might be expected (fig. 1).

The changes, however, were not in direct proportion to the degree of rotation. The electrical axes did not change in proportion to the shift in anatomic axes, even though the change, in most cases, was greater with the higher degree of rotation. This was particularly true for the  $RS$  axes. The change in the  $T$  axes with a given degree of rotation was greater than for  $RS$ . The results of rotation to the right were more consistent and pronounced than in rotation to the left. In the main,

rotation of the heart to the right resulted in a decrease in the amplitude of *S* II and III and of *T* II and III, and the decrease was greatest in *S* II and *T* II, in most cases.

In Lead I, the results obtained depended upon the direction and configuration of *R*, *S*, and *T* before rotation. Where *R* was relatively prominent in I and *T* was negative, rotation to the right resulted in an increased *R* and an increased negative *T*. These changes were in opposite direction to those obtained following rotation to the left. When an *S* and an upright *T* were present in Lead I (one case), before rotation, the *S* was replaced by an *R* and *T* became diphasic, but mainly negative with the higher degree of rotation.

The degree of change in electrical axes for *RS* was greater for rotation right, but was not directly proportional to the degree of rotation of the heart. The degree of change in the *T* axes was of the same magnitude for rotation to the right and to the left.

The results obtained for the chicken heart appear to be in closer agreement with Einthoven's theory than the results of rotation of the heart of dogs, as reported by Meek and Wilson (3), and others. Meek and Wilson showed, in rotating the heart of the dog on its antero-posterior axis, that the heart was also rotated on its longitudinal axis, which affected the results obtained. This factor may have influenced the results obtained on the chicken heart, particularly in rotation to the left. The size and shape of the left ventricle is such that, in rotation to the left, the tendency for the heart to rotate also on its longitudinal axis is greater than in rotation to the right.

#### SUMMARY

The hearts of chickens were rotated on their antero-posterior axes to the right and the left.

In rotation to the left there was a decrease in amplitude of *S* III and II and of *T* II and III, but the decrease was greatest in Lead III. In Lead I before rotation the ECG's of most of the birds exhibited a small *R* wave, no *S*, and usually a negative *T* wave. After rotation the *R* decreased or disappeared and was replaced by an *S* wave, and the *T* wave became positive. The electrical axes for *RS* increased in most instances after rotation and the *T* axes decreased. The degree of change in the axes was not directly proportional to the degree of rotation of the heart, but in most instances the change was greater with the higher degrees of rotation.

Rotation to the right resulted in a decrease in amplitude of *S* II and *S* III and of *T* II and *T* III, but the decrease was greatest in II. In Lead I, the *R* wave and the negative *T*, which were present in most birds before rotation, increased in amplitude after rotation. The electrical axes for *RS* decreased after rotation and the *T* axes increased. The degree of change in the *RS* axes was greater in rotation of the heart to the right than to the left, but the change was not directly proportional to the degree of rotation. In most instances, the change was greater with the higher degrees of rotation.

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# STUDY OF SIMULTANEOUS RIGHT AND LEFT ATRIAL PRESSURE PULSES UNDER NORMAL AND EXPERIMENTALLY ALTERED CONDITIONS<sup>1</sup>

D. F. OPDYKE, J. DUOMARCO,<sup>2</sup> W. H. DILLON,<sup>3</sup> H. SCHREIBER,<sup>3</sup> R. C. LITTLE  
AND R. D. SEELY

*From the Department of Physiology, Western Reserve University Medical School*

CLEVELAND, OHIO

THERE is indirect evidence that in the presence of an interatrial septal defect there is a transfer of blood from left to right atrium. This implies a greater pressure in the left atrium which has been directly demonstrated (1, 2). It has not been shown, however, whether such a pressure difference exists throughout the cardiac cycle or only at specific moments. As a first approach to the study of the hemodynamics of inter-atrial communications it is therefore important to know the normal relation between pulse contours, synchronicity of events and instantaneous pressure relations in the cavities of the two atria.

Although the right atrial pressure cycle and its variations is well-known, that of the left has received only a modest amount of attention, chiefly in connection with studies of pulmonary hemodynamics. The older observations consisting mostly of investigations carried out before the advent of modern optical methods of recording have been reviewed by Tigerstedt (3) and Wiggers (4). A few uncalibrated records of left atrial pressures obtained by modern optical methods have been published by Piper (5), Straub (6), Wiggers (7), Wiggers and Katz (8). Simultaneous recordings of right and left atrial pressures by adequate manometers are exceedingly few and none have been quantitated.

This investigation was carried out with the express purpose of comparing the basic effects which changes in blood flow have on right and left atrial pressures simultaneously recorded. Such studies can be made with greatest accuracy in open chest experiments in which the complicating effect of changes in intrathoracic pressure on venous pressures is abolished. In this way it is possible to establish a basis for comparison of results obtained when interatrial septal defects are experimentally produced. Furthermore, since the problem of hemodynamics in the pulmonary circuit seems destined to be reinvestigated by means of the right heart catheterization technique (9, 10), these observations should also be of use in interpreting the new information so obtained.

## METHODS

Mongrel dogs of average size were adequately anesthetized with 3 mg/kg. of morphine subcutaneously and 180 to 200 mg/kg. of sodium barbital intravenously. Aortic and atrial pressures

Received for publication July 19, 1948.

<sup>1</sup> Supported by a grant from the Life Insurance Medical Research Fund.

<sup>2</sup> Guggenheim Fellow.

<sup>3</sup> Life Insurance Medical Research Student Fellow.

were recorded by optical manometers of the Gregg design. The manometers used for aortic pressure had frequencies of at least 150 per second; those used to record atrial pressures were about 50 to 70 per second. There was no parallax between the various recording beams. Each manometer beam was calibrated in respect to its base line at the end of each record.

A tracheal cannula was inserted, the right carotid artery exposed and a femoral vein cannulated. Later, a cannula for recording aortic pressure was introduced via the right carotid artery so that the tip just reached the arch of the aorta. The approach to the atria was governed by the method of recording the atrial pressure contemplated. A mid-sternal approach with wide retraction of the chest wall was used when pressures were to be recorded via cannulae or semi-rigid catheters inserted through the azygos and/or pulmonary vein, or through the tips of the atrial appendages. In such cases the heart was suspended in a pericardial cradle, care being taken not to impede venous return. The mid-sternal approach was used occasionally when right atrial pressure was recorded by means of a sound passed down the external jugular vein. Usually, when the right jugular sound was employed the left atrium was exposed by resecting the third rib on the left side and left atrial pressure recorded by means of a cannula or catheter introduced via a pulmonary vein or the tip of the atrial appendage. Good records were obtained in a few experiments by resecting the third or fourth rib on each side and recording the atrial pressures through the tip of a 20-gauge hypodermic needle placed in the atrial cavity directly or through the tip of the appendage. Generally speaking, none of these methods has a marked advantage over the others. The rate and volume of artificial respiration was adjusted so that spontaneous respiratory movements just failed to occur. When taking records the respiration was halted for a period of 5 to 10 seconds in order to avoid artifacts and changes in hydrostatic level of the heart due to the inflation and deflation of the lungs.

#### RESULTS AND DISCUSSION

It was necessary in each case to determine the true pressure curve for each atrium and to minimize artifacts caused by intrinsic and extrinsic factors. The true forms of the atrial pressure curves were determined by taking numerous records with different positions and adjustments of the cannulae. Consistency in the form and pressure of a curve with the cannula in several different situations was the criterion of reliability. Records which contained artifacts that appeared to be caused by faulty position of the cannula, e.g., arterial impacts or occlusion during a part of the cycle, were discounted. Some artifacts, however, are present in *all* records. Movement of the heart, arterial impacts and heart sounds set up vibrations which are recorded by sensitive optical manometers. These are unavoidable in most cases if the manometers are of relatively great sensitivity. The analysis of form and instantaneous pressures in regions where they occur can therefore be only approximate.

Reliable records of simultaneous right and left atrial pressures were obtained from 30 animals before starting procedures such as rapid saline infusions, hemorrhage, or stimulation of nerves which could alter the normal relation between right and left atrial pressure. These records have been analyzed with the objective of determining whether any differences exist in the dynamics of blood flow in the right and left atria. Representative segments of normal records from six experiments are reproduced in figure 1 and discussed below.

I. *Pressure Pulse Contour.* The contour of the left atrial pressure pulse is fundamentally the same as the right, exhibiting the same waves but differing in amplitude. In general, the characteristic of the left atrial curve, as recorded by us, is the relatively greater elevation of the 'V' wave, the peak of which roughly coincides with the second heart sound. In most of our experiments the maximum pressure during the left atrial cycle occurred at this point. In this respect our curves differ from

some of those previously published. We attribute this to a more normal venous return than has heretofore been obtained in this type of experiment. The right atrial pressure pulses are typical and need no description.

II. *Synchronicity of Events.* Four easily identified points on the pressure curves were selected for comparison, these points being 1) the beginning of atrial systole, 2) the peak of atrial systole, 3) the end of atrial systole (in reality the beginning of

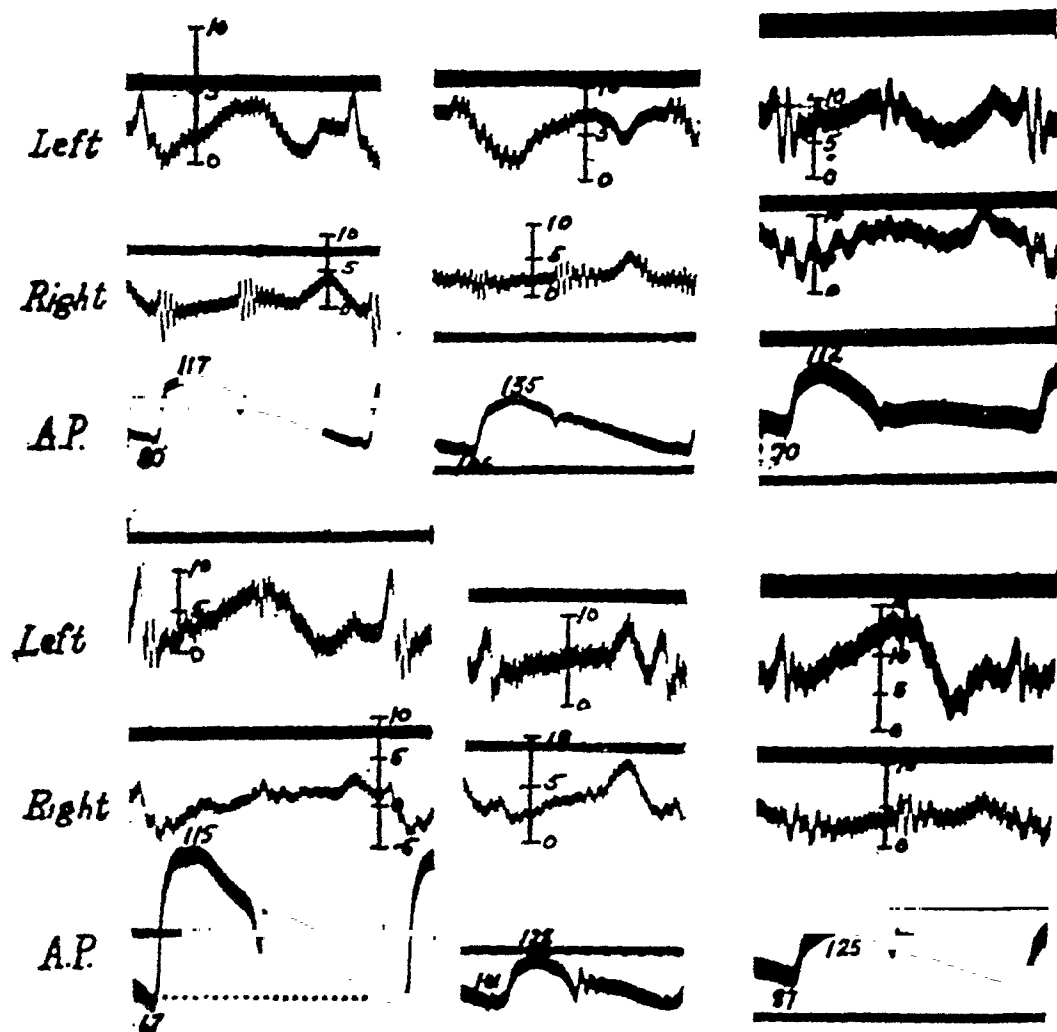


Fig. 1. SIMULTANEOUSLY RECORDED ATRIAL PRESSURE PULSES from 6 representative experiments. Top curve, left atrial pressure; middle curve, right atrial pressure; bottom curve, aortic pressure (A. P.).

ventricular isometric contraction, the so-called 'Z' point), and 4) the second heart sound artifact which we have called the 'V' point. Only records in which these four points could be identified without question on both right and left atrial curves were analyzed.

The results of this analysis are presented graphically in figure 2. No consistent pattern of asynchronicity exists. Right atrial systole may begin as much as 0.03 second before left, but in about 50 per cent of the cases the atrial systoles are simul-

taneous. Occasionally left atrial systole preceded right by 0.01 to 0.02 second. The peaks of atrial systoles may be synchronous or asynchronous in either direction and the precedence bears no relation to the order of precedence of the beginning of the atrial systoles. The end of left atrial systole as marked by the beginning of ventricular isometric contraction frequently precedes right, but this is a ventricular event rather than an atrial one. The appearance of the second heart sound artifact is simultaneous in both curves.

### *Left Atrial Events Referred to Right Atrial Curve*

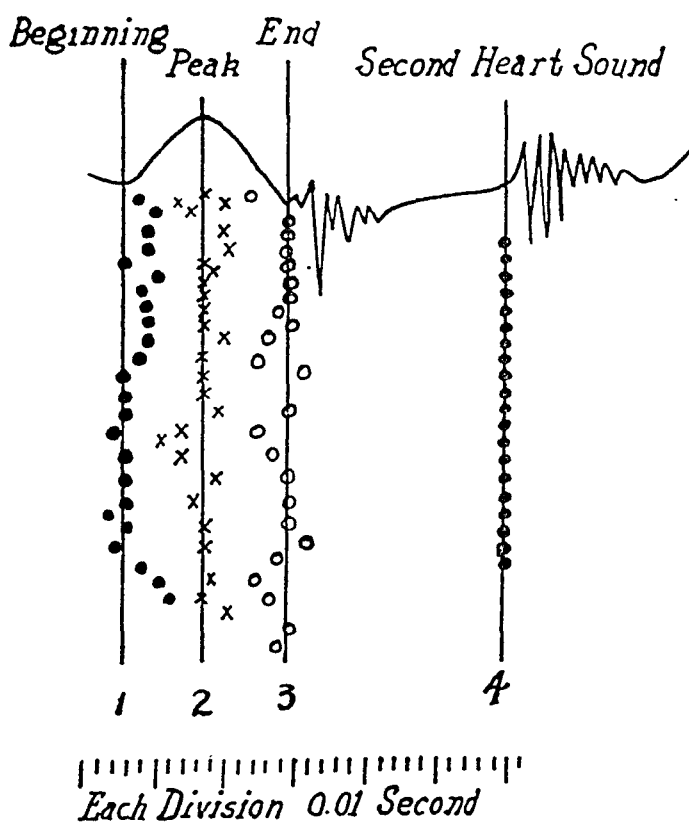


Fig. 2. TIME RELATION between comparable right and left atrial events.

III. *Pressure Relations Between Right and Left Atria.* An important objective of this investigation was to determine what hemodynamic gradients normally exist, or can be produced, between the two atria in the open chest animal that would be of importance in determining the direction and quantity of blood flow through a *theoretical* inter-atrial shunt. A comparison of simultaneously existing right and left atrial pressures throughout the cardiac cycle and under controlled experimental conditions gives us such information.

A. *'Normal' pressure relation.* The size and direction of the pressure gradient existing between the two atria from moment to moment was carefully measured in many experiments before instituting procedures which would experimentally alter the relation. By means of the coordirectograph described by Green (11) differential pressure curves were constructed by subtracting the right from the left atrial pressure curve. The six constructed curves reproduced in figure 3 are representative of all

types encountered. Such curves show the direction and magnitude of the pressure gradient across the inter-atrial septum at each moment of the cycle.

In about 50 per cent of the cases (curves 1-3) the pressure gradient is from left to right atrium at all times during a single cycle. Although the pressure gradient seldom exceeds 5 mm. Hg, it is evident that the direction of blood flow would have been from left to right atrium had an inter-atrial communication been present. This observation adds weight to a clinical impression that such is the direction of flow through congenital shunts in man (1, 2).

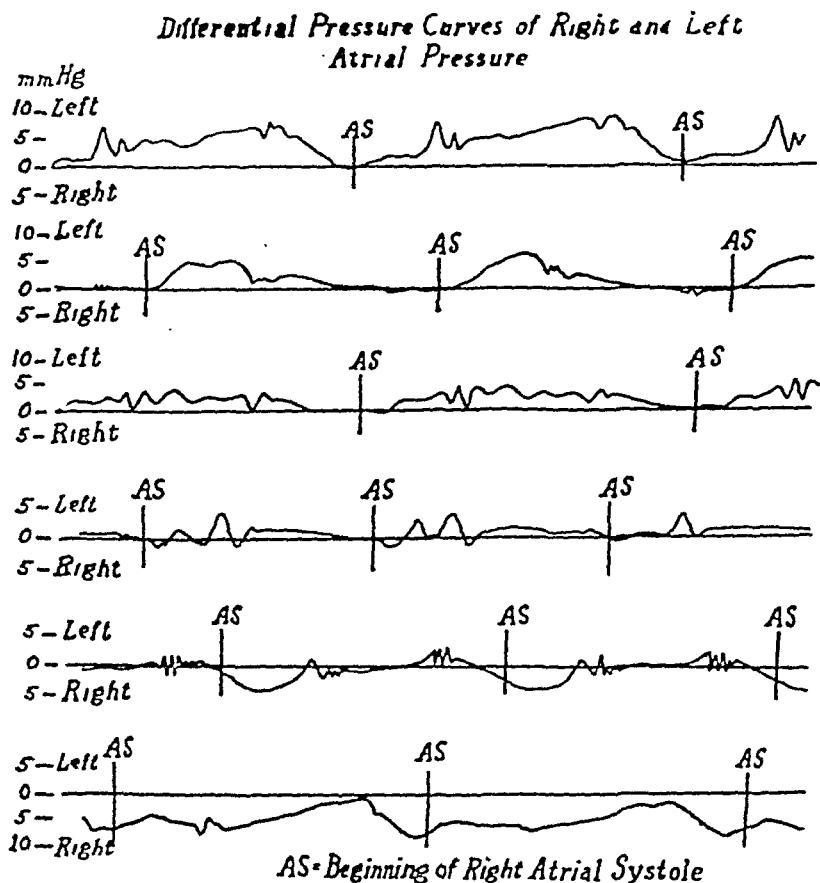


Fig. 3. CONSTRUCTED DIFFERENTIAL PRESSURE CURVES showing the moment to moment difference between right and left atrial pressure. A-S, beginning of right atrial systole.

However, the pressure gradient across the inter-atrial septum is not always from left to right or even constant in direction. In about 40 per cent of the cases of which curves 4 and 5, figure 3, represent the extremes, the gradient was predominantly from left to right, but reversed direction for a brief period (0.1-0.12 sec.) during some portion of the cycle. The reversal of direction sometimes occurred during atrial systole and sometimes during diastole. It did not appear to be associated with the degree of asynchronicity of the atrial events. In most cases, however, the period during which the direction of the gradient was from right to left atrium was very short and the pressure differential very small (1-2 mm. Hg), so we are of the opinion that little blood would have been transferred from right to left atrium had an inter-atrial septal defect been present.

About one dog in 10 exhibited a right atrial pressure that was greater at all times during the cycle than left atrial pressure. A differential pressure curve of one such animal is illustrated by curve 6, figure 3. One of the striking things about such cases is the fact that when this situation is found the pressure differential is considerable, averaging 5 to 12 mm. Hg.

B. *Effect of infusions on atrial pressures.* It was our desire to alter the circulatory conditions in a number of ways in order to observe the effects on atrial pressures. One of the simplest methods of achieving this end is to increase venous return via an intravenous infusion.

TABLE 1. SIMULTANEOUS RIGHT AND LEFT ATRIAL PRESSURES, IN MM. HG DURING INFUSION OF BLOOD

POINT.....	1		2		3		4	
	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
A. Control; A.P. 135/95, C.L. 0.42"	1.0	2.0	3.0	7.5	0.8	6.5	1.0	1.5
B. 50 cc. in; A.P. 155/122, C.L. 0.44"	2.5	6.4	4.0	7.0	2.0	9.0	2.0	4.1
C. 100 cc. in; A.P. 176/135, C.L. 0.46"	4.1	8.0	6.5	11.0	4.5	11.0	4.0	12.0
D. 150 cc. in; A.P. 193/145, C.L. 0.46"	5.5	11.0	9.0	15.0	7.2	15.5	7.0	18.0
TOTAL INCREASE.....	4.5	9.0	6.0	7.5	6.4	9.0	6.0	16.5

Pressures measured at pts. indicated in fig. 2. C.L. = Cycle length. Duration of infusion =  $3\frac{1}{2}$  min.

The effect of moderately rapid infusions of blood or saline into a femoral vein was studied first. The rate of infusion was usually 50 to 75 cc. per minute and the total volume varied between 100 and 500 cc. Simultaneous atrial pressures were recorded and the pressures measured at four different points during each cycle. These points, indicated in figure 2 were: 1) beginning of right atrial systole, 2) peak of right atrial systole, 3) end of right atrial systole, so called 'Z' point, and 4) just before appearance of second heart sound artifact, the 'V' point. Further reference will be made to these points by the appropriate number.

The change in atrial pressure relations as a result of infusion was remarkably constant in a series of 20 experiments. Therefore, the details of one of the best illustrates the typical result. Table 1 summarizes the data obtained from analysis of the optical records. By the time 50 cc. of blood had been infused the pressure in both atria was elevated at the four measured points, with a single exception (point 2 of the left atrial cycle). However, after 100 cc. had been infused the pressure had increased at all points. The striking feature in this case and all others is the fact that left atrial pressure increased more than right and by a considerable amount (see tabulation of total pressure change at each point). The greatest rise in atrial pressure, right or left, occurred invariably at point 4 on the left atrial pressure curve. The fact that the pressure at point 4 rose somewhat more rapidly, particularly after the first 50 cc. of infusion, strongly suggests that the rise of left atrial pressure was



due chiefly to an increase in left atrial inflow and not to back pressure effects occasioned by the rise of aortic pressure since the A-V valves were closed at this point.

We conclude on the basis of these experiments that a larger venous return would result in a greater pressure differential across the inter-atrial septum increasing the gradient from left to right. In the face of such a situation a larger quantity of blood would have been returned to the right circuit had a communication between the atria existed. Furthermore, it would appear that 'reversal effects' (i.e., a drop in systemic arterial oxygen saturation as a result of reversing the direction of flow through the shunt) would not have occurred as a result of infusion. A failure to decrease arterial oxygen saturation as a result of infusion has been reported in a human case with an inter-atrial shunt by Warren and co-workers (1).

The above experiments did not permit us to determine whether the elevation of right atrial pressure always preceded that of the left, or vice versa, since continuous records of infusion were not obtained. In order to clarify this point another series of experiments was performed. Brief rapid saline infusions at the rate of 10 to 50 cc. per second were made directly and alternately into the right and left atria via a right jugular sound or a cannula in the left atrial appendage. Atrial and aortic pressures were recorded continuously so that each record contained control, infusion, and recovery beats. Since the duration of infusion was usually not longer than 5 to 10 seconds it was possible to use a fast speed of the photokymograph, thus allowing the curves to be spread out and the pressure changes at various points of each consecutive cycle analyzed. Atrial pressures were measured at the previously indicated four points.

The changes observed with this type of infusion were consistent in a total of eight experiments. Therefore, the results of only one experiment are presented in detail in figure 4. The rate of infusion in this experiment was 18 cc. per second for a duration of a little over four seconds. As shown in figure 4, left atrial pressure increased considerably more than right at points 1, 2 and 4 during right arterial infusion. The increase appears to be parallel at point 3. Conclusions as to whether left atrial pressure begins to rise before right depend on which point of measurement one considers. Making allowances for random variation in the control pressure, the rise following the beginning of infusion is simultaneous at points 1, 2 and 3, but at point 4 a significant rise in left atrial pressure occurs before any significant increase in right pressure.

The reason for the simultaneous rise of both atrial pressures at points 1 and 2 shown on the first infusion beat is not clear. The increase in left atrial pressure apparently is not due to an instantaneous transmission through the pulmonary circuit. This could not be expected to elevate pressures other than at the V point. But as shown in figure 4 pressures are elevated at all points on the venous curve. This could be explained by a shift of the inter-atrial septum, but this appears to be excluded by the fact that the rise of pressures at the V point is greater on the left.

Infusion into the left atrium of the same dog at the same rate produces an entirely different effect as might be expected. Figure 4 reveals that left atrial pressure increases promptly and to a considerable extent on the first infusion beat. No significant rise in right atrial pressure had occurred at any of the measured points by the twelfth infusion beat, at which time the infusion was terminated. However, the

maximum left atrial pressure level did not exceed that finally achieved when saline was infused into the right atrium except at point 4. It should be noted, however, that control pressure at point 4 in the case of left atrial infusion was greater (4.7 mm. Hg) than the comparable control pressure in the previous case (3.5 mm. Hg). The total pressure increase in the two cases was, therefore, not greatly different.

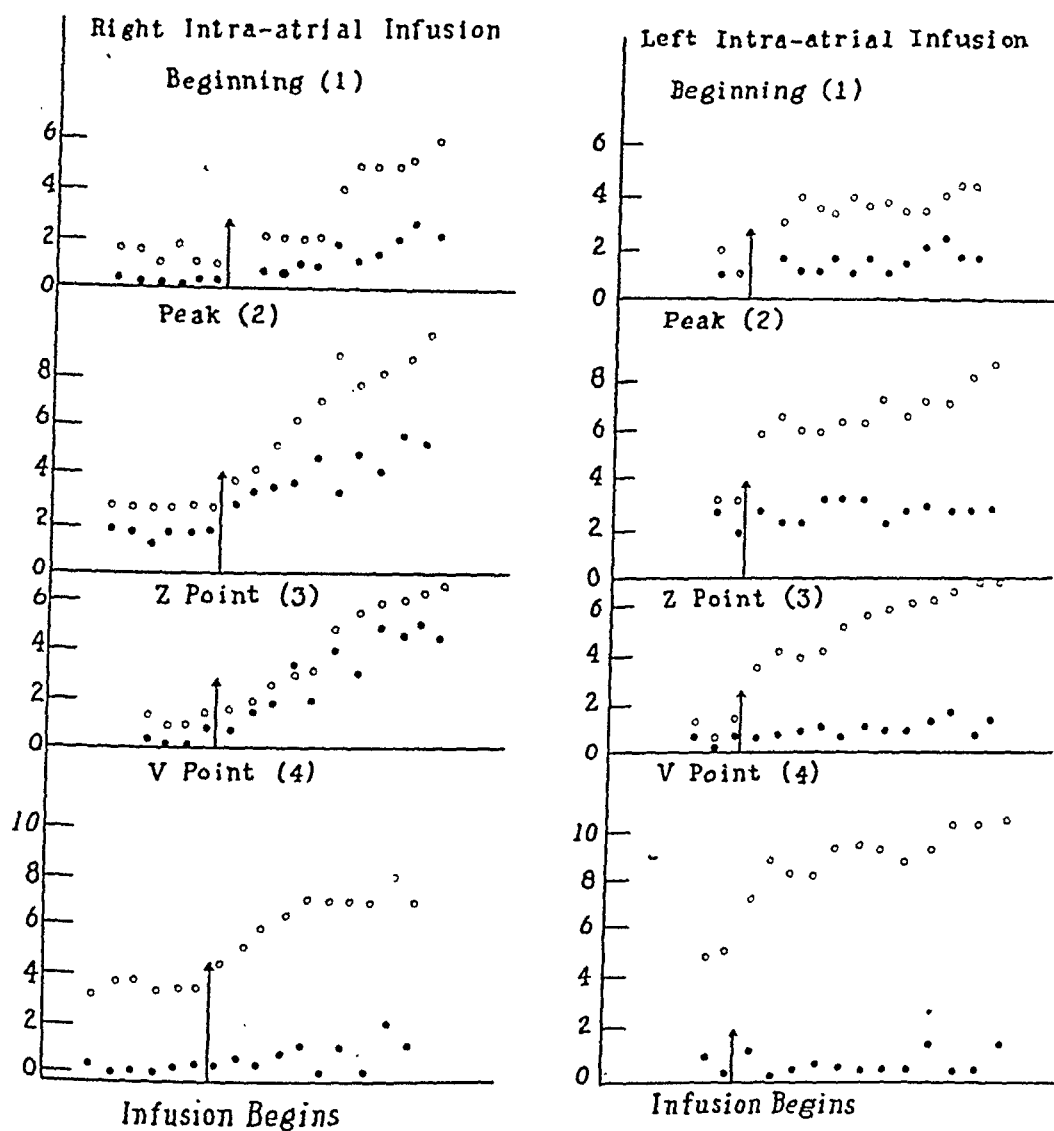


Fig. 4. ATRIAL PRESSURE CHANGES in successive cycles during rapid intraatrial saline infusion. Open circles, left atrial pressure at indicated points; solid circles, right atrial pressure at indicated points (see figure 2). Abscissae, mm. Hg; Ordinates, successive beats.

Little difference in the time at which aortic and pulse pressure began to increase was noted in this or other experiments when saline was infused into the right and left atria alternately. A comparison of systolic and diastolic pressures in consecutive beats during such a pair of infusions (table 2) shows that pulse pressure began to increase not later than the second infusion beat. Since infusion began in the right atrium during early diastole of the fourth control beat tabulated, the aortic pressure showed a significant rise on the succeeding beat. However, in the case of the left atrium, infusion began early in systole so a rise is not noted until the second infusion

beat. For this reason it appears that there is little or no difference in the time at which aortic pressure increases.

The pulse pressure level increased somewhat faster in the case of left atrial infusion but systolic and diastolic pressure did not reach as high a level as when infusion was into the right atrium and pressures tended to fall before infusion was terminated. This is probably explained by the earlier arrival of the saline at the periphery, thus reducing peripheral resistance. The change of pulse pressure in the early infusion beats, perhaps to the sixth beat (at which time the peripheral viscosity factor appears), indicates a marked increase in cardiac output as a result of the intra-atrial infusion.

The failure of right atrial pressure to rise when a very rapid saline infusion is made into the left atrium has several significant features. In the first place it indicates that a rise in left atrial pressure does not reduce right cardiac output through back pressure effects. In all probability right venous return is augmented during

TABLE 2. AORTIC PRESSURE CHANGES OCCURRING AS A RESULT OF INTRA-ATRIAL SALINE INFUSION. INFUSION RATE, 18 CC/MIN. SAME CYCLES AS IN FIGURE 4.

CYCLE NO.....	CONTROL				INFUSION BEATS										
	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11
Syst.....	100	100	97	99	104	108	118	123	139	151	164	165	168	165	
Infusion of Right Atrium															
Diast.....	71	71	68	—	74	77	85	91	99	107	115	115	115	110	
P. P.....	29	29	29	—	30	31	33	32	40	44	49	50	53	55	
Heart rate 162/min. except in beats 8, 9 and 10 where rate = 130/min.															
Syst.....	98	103	102		108	125	136	140	143	145	147	146	140	138	140
Infusion of Left Atrium															
Diast.....	70	72	73		79	90	98	99	100	100	99	95	94	88	87
P. P.....	28	31	29		29	35	38	41	43	45	48	51	46	50	53
No change in heart rate, 160/min.															

the late infusion beats since the changes in pulse pressure indicate an increase in systolic discharge, although this is not reflected by any significant rise of right atrial pressure. In the second place, if it is assumed that right heart output is not decreased by the greater left atrial pressure, then the pulmonary arterial and/or capillary bed must accommodate an increase in volume roughly equal to the infusion volume without backward pressure effects on the right heart. The recent observations of Courmand (9) on the pulmonary bed capacity indicates that this is possible. Thirdly, the magnitude of the atrial pressure rise, particularly at point (4), offers further evidence that an atrial septal shift is not involved in the simultaneous pressure increases noted when saline was infused into the right atrium.

The observation that right intra-atrial saline infusion produces a greater increase of pressure in the left atrium than in the right raised the question of the volume-elasticity characteristics of the atria and their associated great veins. It is evident that the pressures at points 1, 2 and 3 tend to be determined by the venous inflow, by the capacity and elasticity of the atria, large veins and ventricles, since these

are freely communicating cavities. However, at point 4 the A-V valves are closed and the atrial pressures are functions of the inflow, capacity and elasticity of the atria and the great veins. On anatomical ground one would expect that the left atrio-venous system would have a greater volume-elasticity coefficient (more rigid) than the right since it has a smaller capacity, and the left atrial walls appear thicker (not necessarily so in the dog, but apparently so in the human (2)) and at equilibrium the inflow is the same into right and left heart. However, to the best of our knowledge differences in volume-elasticity relations of the two atria have never been demonstrated experimentally in the intact mammal. Our experiments offered an opportunity to at least study this in a semi-quantitative manner. Two different infusion rates were employed, right and left atria receiving an infusion alternately at each rate, sufficient time being allowed for recovery between observations. The increase in atrial pressure at the various points on the same side as the infusion were measured one second after the beginning of infusion and the values plotted against the infusion rate, as illustrated in figure 5. We now have a simple volume-pressure

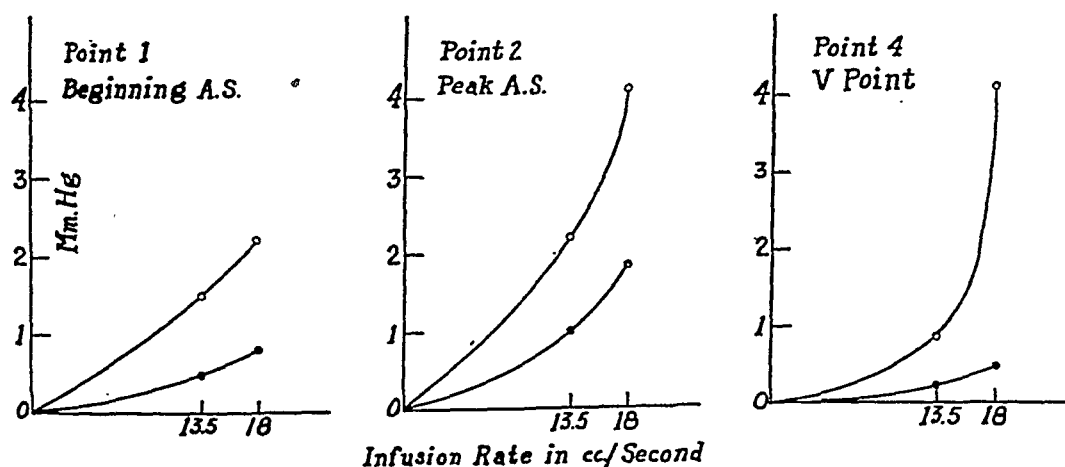


Fig. 5. QUASI-VOLUME-ELASTICITY CURVES of the right and left atrio-venous-ventricular cavities at various points of the atrial cycle.

relation graph in which the pressure change was measured and the volume is a function of the infusion rate (which was constant), plus the animal's own venous return which was assumed to be constant. It is obvious that this is a crude demonstration and that the volume-pressure relation cannot be given in quantitative terms. It does indicate, however, that the left heart is a less elastic structure than the right since the same volume inflow results in a greater left atrial pressure. We are particularly impressed with the restricted distensibility of the left atrio-venous system and, conversely, with the apparently great ability of the right atrio-venous system to store blood without a significant increase in pressure.

C. *Effect of hemorrhage on simultaneously recorded atrial pressures.* Dogs were bled from the femoral artery at the rate of 50 cc. per minute. Both right and left atrial pressures were observed to decrease simultaneously, but the rate of decline was faster for left atrial pressure than for right. The data from one hemorrhage experiment have been assembled in table 3. Atrial pressures were measured at the four usual points. Note that at each point the total decrease in pressure is greater in the left than in the right atrium.

The greater decrease in left atrial pressure than in right will of course decrease the pressure gradient from left to right across the interatrial septum, and if an interatrial shunt were present it would decrease the rate of flow into the right atrium from the left. However, the direction of the gradient would not be reversed until the animal had progressed to a moribund state.

*D. Effect of increased peripheral resistance on atrial pressures.* An increase in peripheral resistance in the intact animal produces a complex series of circulatory adjustments. Theoretically, right venous return should decrease due to vasoconstriction thus reducing right venous return, atrial pressure, and right heart output, resulting ultimately in decreased left heart output. Such a sequence is not observed

TABLE 3. CHANGES IN LEFT AND RIGHT ATRIAL PRESSURES RESULTING FROM HEMORRHAGE. PRESSURES MEASURED AT POINTS INDICATED IN FIGURE 2

POINT	1	2	3	4	CYCLE LENGTH
Atrial pressure	mm. Hg	mm. Hg	mm. Hg	mm. Hg	seconds
Control					
A.P. 135/101 Rt.....	6.0	9.5	2.0	5.2	0.34
Lt.....	7.3	8.0	3.5	8.0	
50 cc. out					
A.P. 124/90 Rt.....	5.5	9.0	1.7	5.0	0.34
Lt.....	6.8	8.2	3.0	6.8	
150 cc. out					
A.P. 97/67 Rt.....	4.8	8.0	1.5	4.5	0.34
Lt.....	5.5	6.3	3.0	4.5	
250 cc. out					
A.P. 57/35 Rt.....	4.5	7.0	1.8	4.0	0.38
Lt.....	4.0	4.0	1.0	4.5	
TOTAL DEC. Rt.....	-1.5	-2.5	-0.2	-1.2	
Lt.....	-3.3	-4.0	-2.5	-3.5	

in the intact animal, however, because the emptying of blood reservoirs and the increased arterial pressure serve to maintain or even increase right venous return and cardiac output may increase (8).

Since increasing peripheral resistance results in such profound circulatory adjustments, it was desirable for our purposes to observe the effect on simultaneously recorded atrial pressures. The increase in peripheral resistance was achieved by stimulation of the central end of the divided left vagus by means of an electrodyne stimulator. Stimulation was continued until the aortic pressure reached a plateau and began to fall.

Segments of a record from one such experiment are reproduced in figure 6. After 18 seconds of left central vagus stimulation (segment B) aortic pressure was elevated, but there was no significant change in left atrial pressure at any of the measured points. Right atrial pressure increased only slightly, the change being 0.5, 2.2, 0.9,

and 0.5 mm. Hg at points 1, 2, 3, and 4 respectively. After 28 seconds of stimulation (segment *C*) aortic pressure reached a maximum with left atrial pressure increasing on an average of 7 or 8 mm. Hg at the respective points. Right atrial pressure showed no significant change from the preceding record, nor did it change appreciably with continued stimulation (95 sec., segment *D*). Following the cessation of stimulation (segment *E*), aortic and left atrial pressures decreased toward control level, but were still somewhat higher than before.

It is obvious that complex circulatory adjustment resulted from the increase in peripheral resistance occasioned by the vagal stimulation. Questions arise as to whether cardiac output increased, whether systemic venous return increased, as to the effect of the slight changes in heart rate on atrial pressures and a myriad of other aspects of the compensation. However, the analysis of these questions is beyond

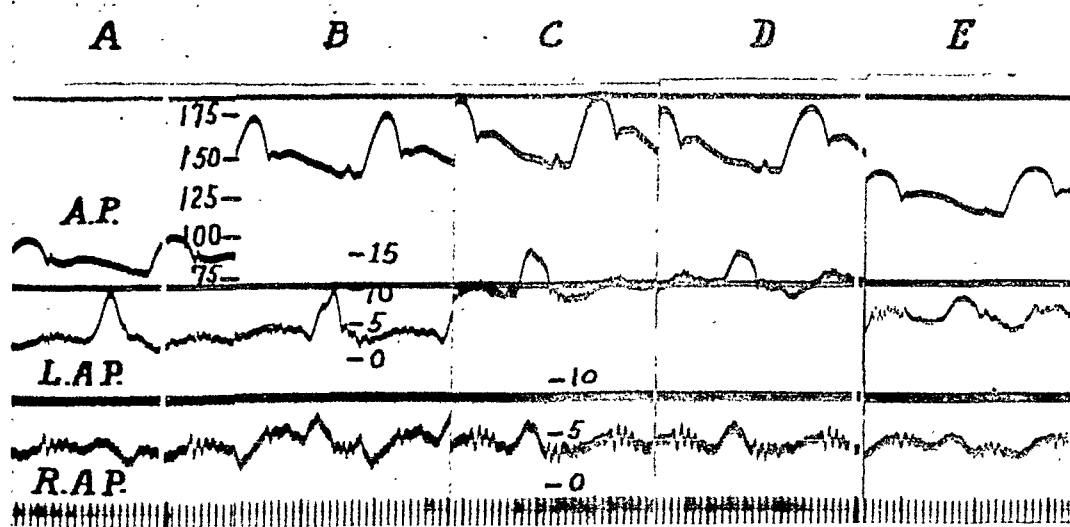


Fig. 6. EFFECT OF CENTRAL VAGAL STIMULATION on right and left atrial pressures simultaneously recorded. A-P, aortic pressure curve; L-A-P, right atrial pressure curve; A, control; B, after 18 seconds of stimulation; C, after 28 seconds; D, after 38 seconds; E, recovery (incomplete).

the scope of this paper. The significant point here is that left atrial pressure increased far more than right, thus increasing the gradient from left to right atrium across the interatrial septum. Had an interatrial shunt been present a rather large quantity of blood would have undoubtedly passed back into the right heart circuit.

*E. Effect of lung inflation on atrial pressures in the open chest dog.* The artificial respiratory rate in the various experiments ranged from 10 to 20 cycles per minute. Some variations of atrial pressure with respiration were observed in all experiments, but the amplitude of these variations was surprisingly small. Both right and left atrial pressure increased slightly with the positive pressure inflation and decreased with the passive deflation. This effect of respiration was observed consistently.

Since the respiratory cycles were rather short in most experiments (ca. 3 sec.), the effect of inspiration and expiration on atrial pressures was intensified by maintaining the lungs in a state of inflation or deflation for a period of five or six seconds and observing the changes in atrial pressure after a new equilibrium had been established. As compared to the pressures during expiration, maintained inspiration invariably caused both right and left atrial pressure to increase, the right much more

than the left, and aortic pressure decreased 10 to 20 mm. Hg with pulse pressure increasing only slightly. Segments of records made during expiration and maintained inspiration are reproduced in figure 7.

The rise in right atrial pressure as shown in figure 7 is usually greater than that of the left. In the case illustrated, right atrial pressure at points 1, 2, 3, and 4 during expiration were 3.8, 6.4, 4.5, and 4.0 mm. Hg respectively, but during maintained inspiration the values were 7.8, 11.2, 7.9, and 9.0 mm. Hg. Comparable left atrial pressures during expiration were 5.0, 8.0, 7.0, and 9.0 mm. Hg and during maintained inspiration 7.2, 11.2, 9.0, and 10.8 mm. Hg. From these experiments it appears theoretically possible that a maintained positive pressure inspiration in an open chest

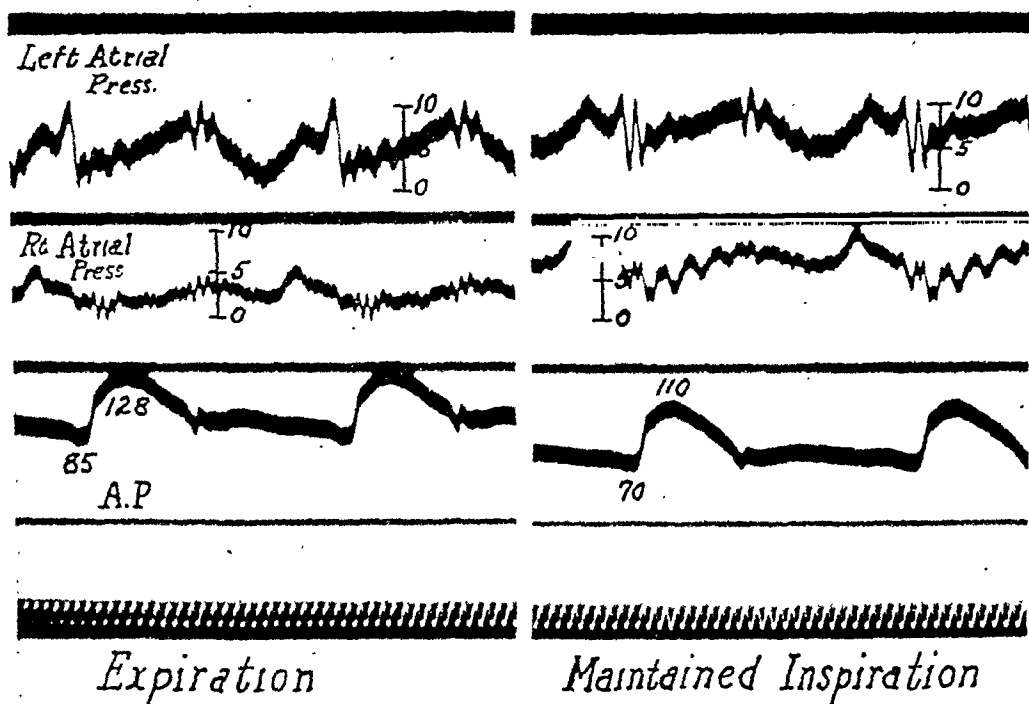


Fig. 7. EFFECT OF LUNG INFLATION (open chest) on right and left atrial pressures simultaneously recorded.

dog would tend to reduce or reverse the flow of blood through an interatrial septal defect. In some instances the right pressure was augmented considerably more during inspiration than in the case used for illustration.

The results of this investigation confirm the numerous reports that left atrial pressure exceeds the right in the dog, although exceptions were noted. However, the question could be asked whether the difference is apparent or real. Since the right atrium is above and partially overlying the left when the dog is in the supine position there is a possibility that the left atrial pressure is greater because of a difference in the hydrostatic level of the atria.

We have demonstrated to our satisfaction that the difference is real by the expedient of filling the open thorax with saline to a depth that covered the beating heart and recording simultaneous atrial pressures while using the upper level of the saline as a common pressure reference level. This obviated any difference in hydro-

static level. The pressure difference existing between the atria was unaffected by this procedure. The greater left atrial pressure must result from a more restricted distensibility of the left atriovenous system and not to gravitational factors as suggested by Uhley (12). When one considers that at equilibrium right and left atrial inflows are equal the finding of a real pressure difference between the atrial pressures confirms a prediction which might have been made on the basis of the volume-elasticity curves previously presented.

Proceeding on the assumption that the left atriovenous system has a greater volume-elasticity coefficient than the right it can also be predicted that a wider range of pressure fluctuations would be encountered in the left atrium. Re-examination of the curves and data reveals that this is true. Left atrial pulse pressure is almost always greater due chiefly to the relatively high pressure created by the atrial inflow during the time the A-V valves are closed. This is in accord with the thesis that the left atriovenous system has a restricted distensibility. The greater fluctuation of left atrial pressure is also demonstrated by alterations in the rate of atrial inflow. Increased inflow (infusion and probably stimulation of central vagus) invariably resulted in a greater elevation of left pressure, whereas decreased inflow (hemorrhage) lowered left pressure more than right. It has been commonly assumed that the range of pressure change in the left atrium parallels that of the right. It is apparent that this assumption is not entirely justified. Care must be exercised, therefore, when making calculations involving pulmonary arteriovenous pressure differences in which the venous pressure change is estimated. There is grave danger that the left atrial pressure change may be underestimated.

If atrial inflow is relatively constant the pressure within the atrial cavity during a single cycle will vary in accordance with the outflow (ventricular filling) and the volume-elasticity characteristics of the atrium. Changes in atrial inflow are, therefore, difficult to judge unless the degree of ventricular filling is known. This difficulty can be empirically obviated if one infers changes in atrial inflow from the change in atrial pressure only at the beginning or end of ventricular filling—the V or Z point. At these stages the outflow from the atrium is zero or negligible and pressure is relatively high. The atrial volume elasticity coefficient is greater and hence an increase in volume inflow will result in a more marked pressure elevation. For this reason it seems preferable to estimate changes in venous return on the basis of a pressure change at a single point of the atrial cycle rather than from changes in mean atrial pressure alone. It has been our experience that the major deformation of atrial pressure curves by artifacts occurs during the time of ventricular systole. When only mean atrial pressures are calculated such deformation may be sufficient to mask significant changes in atrial pressure during other portions of the cycle. This is particularly important when dealing with right atrial pressure, since the volume elasticity coefficient is low and only a slight change in pressure results from a relatively large change in inflow.

Two independent investigations (1, 2) have shown left atrial pressure to be greater than right in the presence of an interatrial septal defect in human subjects. Unfortunately, normal left atrial pressures have not been determined in the human for obvious reasons. Therefore, it is difficult to say whether or not these high left atrial pressures should be considered pathological. However, the observation of a



left to right atrial pressure differential in the open-chest dog is tenuous evidence that the high left atrial pressure observed in humans with interatrial septal defects is not entirely pathological. An incomplete series of experiments on dogs in which interatrial septal defects have been experimentally produced indicate that the pressure differential continues to exist. The observation that increased venous return always augments left atrial pressure to a greater extent than right negates the possibility of using infusion as a means of decreasing the pressure difference between the atria. This is a possible explanation for the failure of rapid infusion to reverse the direction of flow through an interatrial septal defect in a human patient as has been reported (1).

#### SUMMARY

Right and left atrial pressure pulses have been simultaneously recorded in thirty open chest dogs. No consistent synchronicity or asynchronicity between comparable events in the two atria was observed. Left arterial pressure is usually greater than right during the entire atrial cycle, but the direction of the pressure gradient across the interatrial septum may reverse during a cycle.

The pressure relation between the atria was also studied under various circulatory conditions. Rapid blood or saline infusion always increased left atrial pressure more than right, as did central vagus stimulation. Hemorrhage decreased left atrial pressure more than right. The existence of a pressure gradient and the variations thereof is due to a difference between the volume elasticity characteristics of the atria, the left atrio-venous system being less distensible than the right.

Changes in atrial inflow are most correctly inferred from changes in atrial pressure just at the beginning or end of ventricular filling. The atrial volume elasticity coefficient is greatest at these points since the A-V valves are closed and a relatively small change in inflow will produce a relatively large change of atrial pressure.

The greater volume elasticity coefficient of the left atriovenous system accounts for the greater left atrial pressures found in cases with interatrial septal defects and indicates that the elevated left atrial pressure is not entirely pathological. Furthermore, the more restricted distensibility of the left atrium accounts for the failure of infusions to reverse the direction of blood flow through the shunt.

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# DYNAMIC EFFECT OF INSPIRATION ON THE SIMULTANEOUS STROKE VOLUMES OF THE RIGHT AND LEFT VENTRICLES<sup>1</sup>

ROBERT D. SEELY<sup>2</sup>

*From the Department of Physiology, Western Reserve University Medical School*

CLEVELAND, OHIO

THE dynamic effects of inspiration and expiration on the systolic discharge of the right and left ventricles have been frequently studied with contradictory conclusions. According to one viewpoint, the act of inspiration increases venous return and elevates effective right atrial pressures both in animals (1) and in man (2). It is believed that this causes a larger stroke volume of the right ventricle (3, 4). However, the larger discharge is more than accommodated by the increased capacity of the pulmonary bed which results from lung inflation. The increased capacity of the pulmonary vascular bed during inspiration is believed to effect an accumulation of blood in the lungs and to reduce the flow to the left heart. Consequently, effective left atrial pressure decreases, left ventricular discharge diminishes, and aortic pressure declines.

According to another view, inspiration is without effect on venous return or effective auricular pressure. Consequently the stroke volume of the right ventricle remains nearly constant. The elevation in effective pulmonary arterial pressure is attributed to an increase in pulmonary resistance brought about by lung inflation. In support of this view are the recent observations of Duomarco (5) that similar rise of pulmonary arterial pressure occurs during lung inflation when the output of the right heart is kept constant by artificial means. Furthermore, according to Visscher (6), the increased capacity of the pulmonary vessels observed when the lungs are inflated may not take place when the head of pressure as well as the lungs are inclosed in a chamber in which the varying negative pressure can be reduced. Under these more physiologically correct conditions the pulmonary vascular bed is said to decrease and the resistance to increase. The inspiratory decline of aortic pressure recorded against a constant atmospheric pressure is generally interpreted as a transmission of the decreasing intrathoracic pressure to the aorta.

## METHODS

This investigation was undertaken to evaluate simultaneously changes in right ventricular stroke volume by meticulous measurement of effective right atrial pressure and alterations in the stroke volume of the left ventricle by a careful study of aortic pressure pulses. Observations were made during natural breathing and deep breathing following vagotomy in dogs. Dogs were anesthetized with morphine and

Received for publication July 19, 1948.

<sup>1</sup> Supported in part by a grant from the Life Insurance Medical Research Fund.

<sup>2</sup> Present address: Medical Department Field Research Laboratory, Fort Knox, Kentucky.

sodium barbital. Aortic pressure was recorded by means of a Gregg manometer connected to a sound introduced through the carotid artery and right atrial pressure by a similar connection to a jugular sound. Since intrathoracic pressure apparently varies in different portions of the chest (7, 8), an intrathoracic cannula connected to a Frank segment capsule which could be standardized against a water manometer was thrust through the chest wall in the third left interspace so that its tip lay in close proximity both to the tip of the right auricle and root of the aorta. As indicated in figure 1, the technique employed was that of Wiggers, Levy, and Graham (8) who compared pressure relations in several regions of the chest, but not in the space

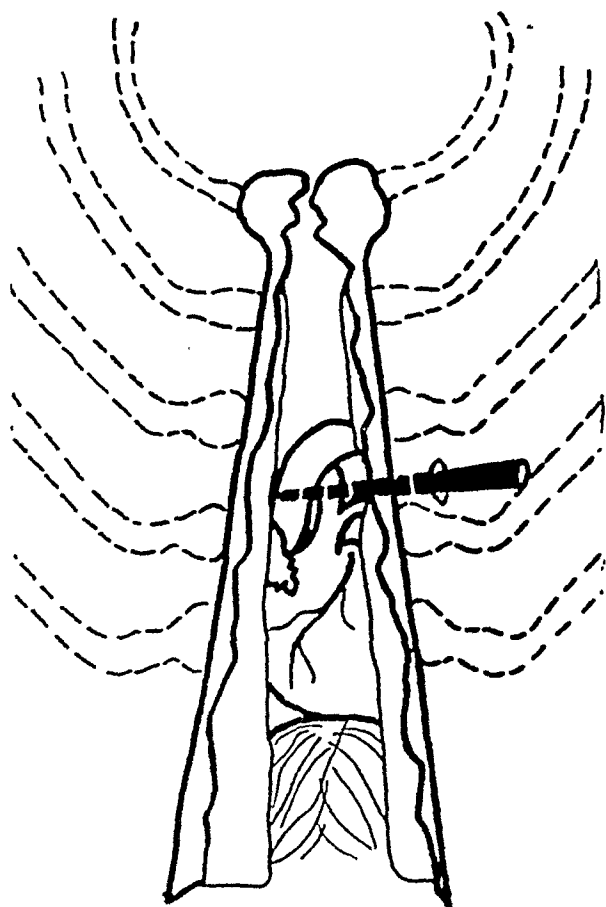


Fig. 1. POSITION OF INTRATHORACIC CANNULA in relation to right auricle and aorta.

surrounding the right atrium and aorta. Once in position, a cavity was created by simply rotating the flat-tipped cannula in various directions until a smoothly recorded curve with superimposed variations was obtained. The location of the cannula was verified by post-mortem examination in all experiments. In this way the true pressure differences between the interior and exterior of the right atrium and aorta could be determined at every moment of the cardiac and respiratory cycles.

#### RESULTS

Illustrative records are shown in figure 2. Records A and B were obtained from naturally breathing animals; records C and D, during prolonged deep inspiration resulting from vagotomy. It will be seen at a glance that the cardiac variations of

intrathoracic pressure in the space around the right atrium and aorta in acceptable records are characterized by a significant diminution during atrial contraction and a slight elevation during ventricular systole. (Fig. 2A, marked 1 and 2). Therefore, in determining effective atrial and aortic pressures at any point of the cardiac cycle simultaneous values of intracardiac pressures must be utilized.

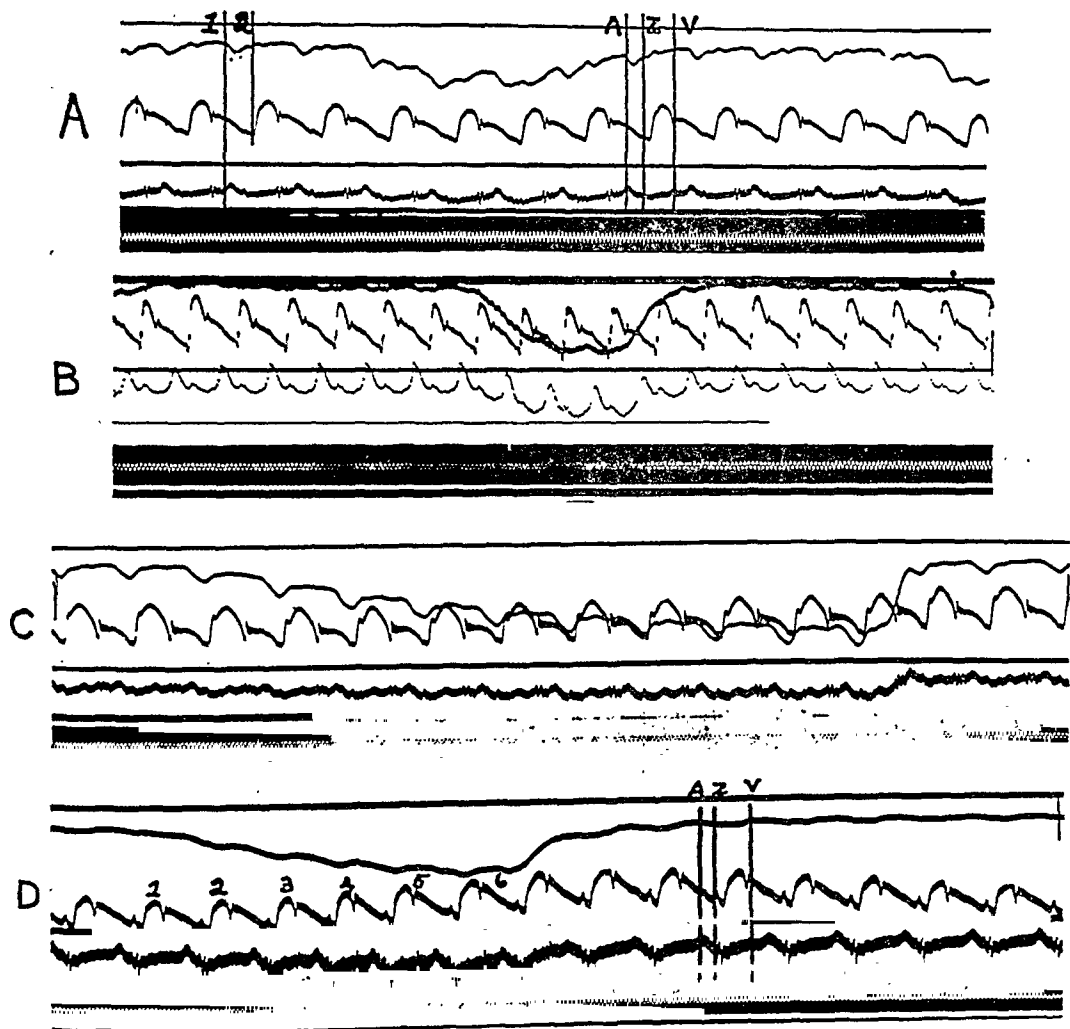


Fig. 2. FOUR RECORDS showing successive changes in intrathoracic, aortic, and right atrial pressures during inspiration and expiration; *A* and *B* during natural breathing; *B* and *C* after vagotomy. Time in .02 second. About  $\frac{3}{4}$  actual size. *A*, lines 1 and 2 showing changes of pressure around atrium and aorta due to atrial and ventricular contractions. Lines *A*, *Z*, and *V* show points at which corresponding pressures were measured at peak of atrial systole, at onset of ventricular contraction and at beginning of ventricular filling.

In order to obtain a complete analysis of effective pressure variations the atrial component was measured at three points of the cardiac cycle, viz., 1) the peak of atrial systole, 2) the beginning of ventricular isometric contraction and 3) the point of maximal atrial volume. These points are marked *A*, *Z*, and *V*, respectively, on one of the records of figure 2. Simultaneous points on the intrathoracic pressure curve were then measured and the effective pressure calculated as the algebraic difference of these two values. Such measurements of records from 10 experiments yielded the following results. In three, atrial pressure showed no measurable difference;

in three it decreased a trifle more during inspiration (ca. 1 mm. Hg) than extracardiac pressure, i.e., effective pressure was reduced a trifle. The reduction in effective pres-

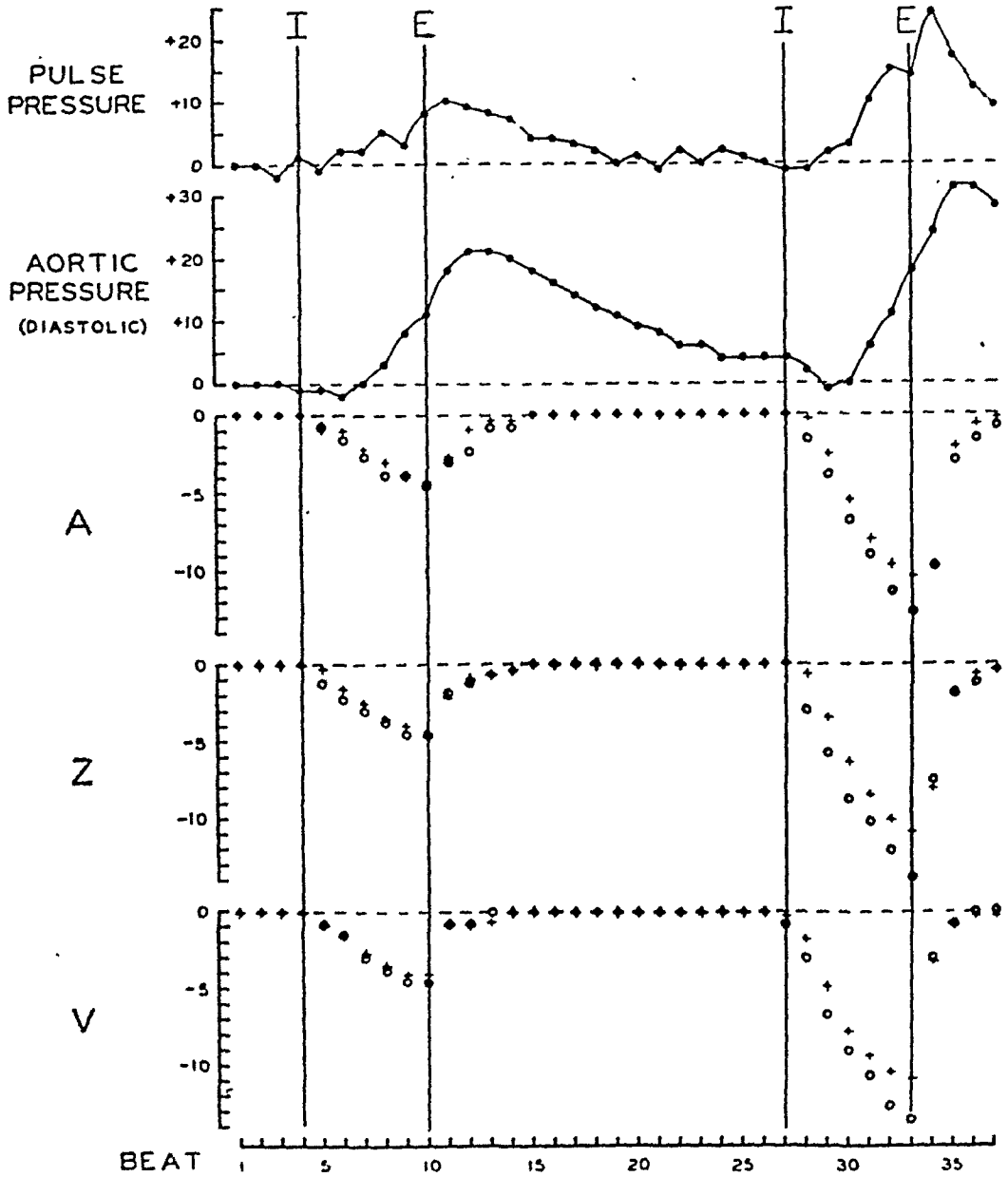


Fig. 3. RELATION of pulse pressure and aortic diastolic pressures to right atrial pressure and extracardiac pressure measured at corresponding A, Z and V points. In lower three records: open circles = atrial and crosses = extracardiac pressures. Changes expressed in mm. Hg from control expiratory beat.

Two respiratory cycles are shown; the first after vagotomy, the second with a mild tracheal occlusion during inspiration. I, onset of inspiration; E, of expiration.

sure was slightly greater at the peak of inspiration after vagal section. In four experiments a slight increase in effective pressure occurred during inspiration, but again this was of small magnitude (1 mm. Hg or less). In only one instance following vagal

section was an increase of effective atrial pressure of 3.7 mm., 2.2 mm., and 3 mm. Hg noted at the *A*, *Z*, and *V* points, respectively.

Figure 3 shows a plot of the relative pressure changes within and around the right atrium as measured at the *A*, *Z*, and *V* points during a deep inspiration and expiration. The very close correspondence between changes in intra- and extra-atrial pressures is remarkable. During the second respiratory cycle plotted the tracheal tube was slightly occluded during inspiration only. This has the interesting effect of reducing intra-atrial somewhat more than extracardiac pressure, decreasing rather than increasing effective venous pressure.

TABLE 1. CHANGES IN AORTIC DIASTOLIC PRESSURES (EXPRESSED IN NUMERATORS) AND EXTRA-AORTIC PRESSURES (MINUS QUANTITIES IN DENOMINATORS) DURING CONTROL BEAT IN EXPIRATION AND IN CONSECUTIVE INSPIRATORY BEATS

EXPT.	EXPIRATION CONTROL BEAT	INSPIRATION BEAT NO.					
		1	2	3	4	5	6
I-4	150/-4.1	147/-5.8	147/-7.8	148/-7.9			
I-5	103/-4.8	102/-5.7	101/-9.8				
2-2	86/-13.5	76/-18.5	74/-23.				
2-9	116/-11.5	114/-11.2	109/-14.6	105/-16.2	108/-16.4	109/-16.6	
3-6	103/-6.9	101/-7.3	96/-8.7	89/-16.4	85/-14		
4-3	113/-3.9	112/-4.1	107/-4.8	106/-6.1	106/-7.0		
5-4	90/-6.4	88/-8.9	83/-11.1	86/-11.2	85/-11.2		
6-5	100/-4.6	91/-8.4		94/-9.1	93/-10.8	97/-11.4	97/-14.6
7-2	108/-6.9	107/-6.9	106/-7.9	105/-8.7			
7-4	118/-6.0	117/-6.9	117/-7.4	118/-8.6	119/-9.1	120/-9.1	129/-11.2
8-2	109/-5.8	108/-6.0	105/-7.1	102/-8.6	106/-8.8		
8-6	96/-5.9	94/-7.2	93/-8.4	99/-9.1	100/-9.8	107/-10.2	
9-6	88/-6.4	87/-6.9	86/-8.0	85/-9.1	86/-10.2	87/-11.0	
10-4	94/4.7	90/-5.8	89/-7.9	88/-8.0			

All figures in mm. Hg.

Many observations such as these have failed to show that the act of inspiration has any effect in drawing blood into the thorax or in altering the systolic discharge of the right ventricle.

Since the intrathoracic cannulae also overlay the aortic region it was possible to determine the degree of correspondence between changes in diastolic-aortic pressure and extra-aortic thoracic pressures at the same moment. Obviously this needed to be done in experiments in which the heart cycle was absolutely constant. This was possible in a number of barbital anesthetized dogs, but in most instances could only be accomplished by vagus section. In the simplest types of records, one of which is illustrated in figure 2A, systolic and diastolic pressures decrease during inspiration, the latter slightly less than the former, i.e., the pulse pressure decreases a little. Calculations of the three inspiratory beats revealed that the decline of diastolic pressure compared to a preceding normal beat equaled 4, 5 and 6 mm. Hg respectively. The corresponding extra-aortic pressures for the same three beats changed much less, being equivalent to 1.1, 3.2 and 3.3 mm. Hg. This relationship was rather con-

sistently found in all experiments during the initial portion of the inspiratory cycle (table I). Obviously the decline in diastolic aortic pressure is greater than the reduction in extra-aortic pressure and the latter cannot be used to give a quantitative estimate of the change in intrathoracic pressure around this vessel or the right atrium.

When inspiration becomes deeper and prolonged after vagal section quite a large number of beats occur during this cycle, as shown in figure 2C. In such a case the diastolic pressure decreases during the first two or three beats only; thereafter it rises and the pulse pressure becomes even greater than in control expiratory beats. A detailed plot of numerical values showing the magnitude of changes in diastolic pressure compared to corresponding alterations in aortic pressures is shown in the plot of figure 3. This plot also reveals that the augmentation of diastolic and pulse pressures is carried over into the phase of expiration and only slowly returns to control levels during the subsequent phase of respiratory apnea.

Since the configuration of aortic pressure pulses is not changed, such alterations in pulse pressure may safely be used as an index of directional changes in systolic discharge of the left ventricle. According to such a criterion it seems apparent that the systolic discharge is slightly decreased during the first two or three beats of an inspiratory act, but if this is prolonged the systolic discharge becomes progressively greater and exceeds the control.

#### DISCUSSION

The results obtained by comparing instantaneous pressures in regions around the right atrium and aorta with pressures within these portions of the circulatory system are considered important from a number of angles.

Under normal conditions and an absolutely regular cardiac rhythm the inspiratory changes of instantaneous pressures in the right atrium measured at the summit of atrial systole (*A*), onset of ventricular contraction (*Z*), and beginning of ventricular filling (*V*) are identical with pressure changes in the surrounding intrathoracic space. However, this has been demonstrated only in dogs during natural respiration and under normal conditions; it must be applied with caution to man in abnormal conditions. On the contrary, the changes of intra-aortic pressure are by no means a reliable criterion of changes in pressure around the right atrium and aorta and may not be used in calculations of changes in effective venous pressure.

An analysis of changes in right and left ventricular stroke volumes during inspiration based solely on probable changes in effective right atrial pressures and changes in aortic pulse pressures leads to the following conclusion: When not more than three cardiac beats occur during inspiration, right ventricular filling and presumably systolic discharge remain unaffected; but the left side stroke volumes are reduced. This explains the reduction in pulse pressure and fall in diastolic pressure which is greater than the decline in extra-aortic pressure. This combination of dynamic events favors the view that blood is stored in the lungs during early inspiration.

When more than three heart beats occur during inspiration, aortic diastolic, systolic, and pulse pressures all increase, even considerably beyond apneic control

levels, as shown in figure 2C and D. It is difficult to interpret these changes otherwise than that the systolic discharge during the fourth to sixth inspiratory beats are increased much more than the stroke volumes of the first three inspiratory beats were decreased. The net effect is an increase in cardiac output during inspiration. However, the effective right atrial pressure changes (fig. 3) offer no evidence that the output of the right ventricle is changed at all during prolonged deep inspiration. One of two possible deductions can be drawn; either the pulmonary circuit yields an additional supply of blood to the left heart through mechanical effects of lung inflation or changes in effective right atrial pressures, even when meticulously measured, do not offer a trustworthy criterion of inspiratory changes in venous return which are sufficient to affect right ventricular filling and discharge and, after a delay, the left atrial filling and left ventricular discharge. No new evidence was obtained as to which of these mechanical factors operates solely or dominantly. However, recent studies (9) have shown that owing to the large capacity of the atrio-caval system small changes in venous return may not cause measurable differences in right atrial pressures although left atrial pressures are affected. Since it is difficult to visualize any scheme of pulmonary mechanism by which blood is retained during the early phase and moved forward during a later phase of prolonged inspiration, it appears probable that alterations of venous return and right ventricular output are responsible, even though right atrial pressure changes are not detectable.

#### SUMMARY

1. A technique is described by which changes in right atrial and aortic pressures during inspiration and expiration can be compared with simultaneous changes in intrathoracic pressure immediately around these structures.
2. Calculations of effective right atrial pressure at three moments of the cardiac cycle—height of atrial contraction, beginning of ventricular systole and onset of ventricular filling—failed to reveal evidence that the act of inspiration augments the return of venous blood and right ventricular input.
3. However, this inference must be tempered by a realization that a fair increase in venous return may occur without a measurable increase in effective venous pressure (9). That this occurs also during inspiration is strongly suggested by coincident registration of other dynamic changes. When six or more heart beats occur during a long deep inspiration, such as follows vagotomy, aortic, diastolic, and pulse pressures increase after the third beat of an inspiration, while effective right atrial pressures remain unchanged. It is difficult to conceive of a type of pulmonary mechanism whereby blood is apparently retained during the early phase of inspiration and larger quantities are moved toward the left side during late inspiration,—all without any change in stroke volumes of the right ventricle.

The conclusion follows that failure to detect measurable differences of effective right atrial pressure does not necessarily preclude the occurrence of changes in right ventricular filling and discharge during inspiration.

The writer desires to express to Dr. C. J. Wiggers his gratitude for supervision of the experimental work and for aid in preparing the manuscript.



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# CARDIODYNAMICS OF EXPERIMENTAL INTERVENTRICULAR COMMUNICATIONS<sup>1</sup>

WILLIAM H. DILLON AND H. SCHREIBER, JR.

*From the Department of Physiology, Western Reserve University Medical School*

CLEVELAND, OHIO

NATURAL defects of the interventricular septum caused by developmental arrest are unquestionably accompanied by slow compensatory functional reactions on the part of the two ventricles. Their nature can only be inferred from autopsy studies. However, such interpretations may apply to end results caused by decompensations rather than to true dynamic consequences of the lesion *per se*.

For this reason the immediate dynamic alterations following an artificial communication between the ventricles of mature dogs was studied with the hope that the dynamic alterations may be applicable to defects which are produced naturally. A large external shunt rather than a surgical fenestration of the septum was used for two reasons: 1) surgical defects comparable in size to those that occur naturally are difficult to produce without involvement of the conduction system and consequent alteration of the dynamics of the heart beat; 2) immediate and sequential dynamic alterations can be better evaluated when a communication exists which can be alternately opened and closed.

## METHODS

Dogs under morphine-barbital anesthesia, weighing from 10.5 to 20 kilograms, were subjected to the following surgical procedures: cannulation of femoral vein, trachea, carotid artery and mid-sternal exposure of the heart. The heart was cradled in the pericardium, care being taken not to embarrass venous return or heart action. Interrupted positive air pressure was used for artificial respiration.

Shunts of numerous designs and sizes were tested. The one finally found to be most suitable is shown in figure 1. It consisted of two brass cannulas of 7 mm. bore, the one for the left ventricle being slightly longer than the one for the right. These were connected by a short segment of heavy rubber tubing. A strong hemostat on the tubing served to open and close the communication.

Intraventricular pressures were recorded by calibrated manometers of the Gregg type. In early experiments, number 16-gauge needles connected by flexible lead tubing to the manometer were thrust through the walls of the ventricles at points on the heart which were observed to show the least movement during contraction and relaxation. In later experiments they were combined with the shunt, as shown in figure 1. Aortic pressure pulses were similarly recorded by a sound passed down the carotid artery. Heparin (0.4 cc/kilo of a 1 per cent solution initially, followed by 0.5 cc. every half hour) was used intravenously to prevent clotting.

After control pressures had been recorded simultaneously from both ventricles by hypodermic needles inserted through the ventricular wall, the interventricular shunt was inserted in the following manner: The needles were removed from the ventricular walls and long-jawed, thin scissors inserted

Received for publication July 19, 1948.

<sup>1</sup> This work was done during the tenure of a Life Insurance Medical Research Student Fellowship.

into the needle holes. By opening the jaws, the superficial sinospiral fibers were separated until a hole large enough to admit one end of the external shunt was obtained. One cannula completely filled with saline was first pushed into the left ventricle. A hemostat clamped on the heavy rubber tubing kept blood from escaping from the left arm of the shunt, while the right cannula was being inserted. The two arms were then immediately joined by the rubber tubing and connections with manometers and needles were made.

The effects of two types of experimental maneuver were recorded. In one, simultaneous right and left ventricular and aortic pressures were recorded and the shunt closed during the period of registration. In the other the shunt was opened during the recording period. Records of stabilized effects were taken one to two minutes later. Pressure changes described below were calculated from the averages for the ten beats immediately before and after the opening or closing of the shunt. It was not possible to determine the changes in the first or second beats after the experimental maneuver because of artifacts and occasional extrasystoles induced by the manipulation of the hemostat. Each record thus consisted of three parts: 1) control, 2) the opening or closing of the shunt including at least the next 10 beats and 3) a record taken one to two minutes after the experimental maneuver. This procedure did not prove universally successful. Of 26 experimental animals, 12 fibrillated during the insertion of the shunt. One heart suddenly became hypodynamic, but did not fibrillate. Of the remaining 13 successful experiments 4 were discarded because of the poor contours of the curves

## RESULTS

The changes in pressure pulses from the left and right ventricles and the aorta after opening and closing the shunt are illustrated in figure 2 by one of the records from the nine good experiments.

Comparison of segments *A* and *B* reveal that opening of the shunt elevates initial intraventricular pressure ( $9 < 11$  mm. Hg) and the maximal pressure ( $27 < 34$  mm. Hg) in the right ventricle. The initial pressure in the left ventricle also rises ( $4 < 7$  mm. Hg), but the pressure maximum declines ( $77 < 64$  mm. Hg). The obvious reductions in systolic, diastolic, and pulse pressure in the aorta indicate that the systolic discharge of the left ventricle was reduced. The heart rate slowed a little ( $154 > 149$ /min.).

In order to restore the stroke volume of the left ventricle and the aortic pressures approximately to normal levels, a continuous slow infusion of warm saline was given in this experiment. The improvement which resulted while the shunt remained open is illustrated in segment *C*. The shunt was then closed with the effects depicted in segment *D*. Comparison of these two records reveals that closure of the shunt reduces the initial tension ( $12 > 8$  mm. Hg) and the maximal pressure ( $34 > 29$  mm. Hg) in the right ventricle, while it increases the pressure maximum in the left ventricle ( $81 < 98$  mm. Hg), but causes no measurable change (8 mm. Hg) in the initial pressure. The heart rate increased slightly ( $151 < 153$ /min.).

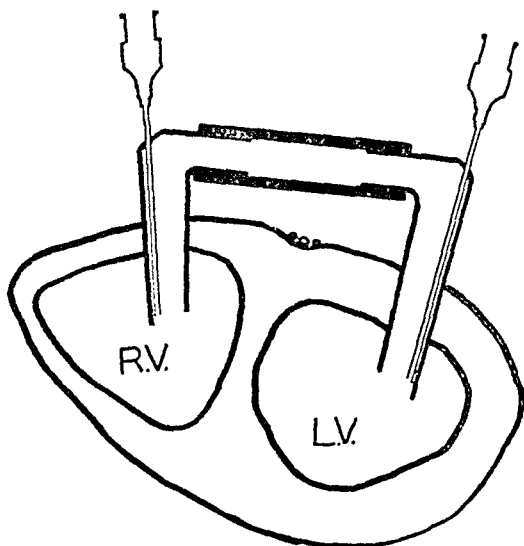
A total of 46 records from nine good experiments similar to those of figure 2 was measured and the results tabulated. This included data concerning changes in heart rate, initial and maximal pressures in the left and right ventricles during control states, immediately and some time after opening the shunt and immediately and after reclosing the shunt. In addition, the duration of systole and its relation to cycle length (S/C ratios) were measured. Changes in aortic pressure pulses were evaluated.

*Initial and maximal ventricular pressures.* The immediate effects of opening and closing a shunt on right and left ventricular pressures are schematically summarized

by the diagrams of figure 3. In 18 records *opening* the closed shunt caused the following immediate effects: In the left ventricle maximal pressures decreased in all cases from 2 to 24 mm. Hg; initial pressure fell in one record, remained the same in 7 and increased in 10, the change varying from  $-1$  to  $+3$  mm. Hg. In the right ventricle maximal pressures increased in all cases from 2 to 17 mm. Hg; initial pressure fell in no case, remained the same in 7 and increased in 11, the change varying from 0 to  $+6$  mm. Hg.

In 15 records *closing* the open shunt produced the following changes: In the left ventricle maximal pressures increased in all cases by 5 to 20 mm. Hg; initial pressures fell in four cases, remained the same in eight and increased in one, the change varying from  $-1$  to  $+1$  mm. Hg. In the right ventricle maximal pressures decreased in all cases by 3 to 15 mm. Hg; initial pressure fell in 3 cases, remained unchanged in 7 and increased in 3, the change varying from  $-2$  to  $+1$  mm. Hg.

Fig. 1. DIAGRAM illustrating the principle of an interventricular shunt and the registration of intra-ventricular pressures.



*Heart rate and systole/cycle (S/C) ratios.* In half of the experiments opening and closing of the shunt produced no changes in heart rate. In the others a slight increase occurred during opening and a slight decrease during closing of the shunt. Since these changes needed to be taken into account in determining the effect of interventricular septal defects on the duration of right and left ventricular contraction, the well known expedient of determining systole/cycle ratios (S/C ratios) was used. Opening of the closed shunt generally caused the S/C ratio to increase in the left ventricle; in other words, contraction was prolonged with respect to cycle length (fig. 4, A—C). Although the peak of systolic pressure came earlier in the cycle (fig. 4, A—B), the incisura came somewhat later and the result was a greater duration of systole. While the increase in the S/C ratio was not found in every record, in the majority of experiments the ratios indicated a prolongation of contraction by 5 to 10 per cent. The changes in S/C ratios generally paralleled alterations in initial tension. In these experiments closing the shunt caused the S/C ratio to shorten. Opening the shunt caused a more variable change in the right ventricle. Three experiments had records which showed an abbreviation of the S/C ratios, although other curves in the same experiments showed the expected increase (fig. 4). Other

experiments analyzed showed the S/C ratio to increase consistently when the shunt was opened.

The onset of systole preceded in the left ventricle when the shunt was closed in six experiments (fig. 4). Precedence of the left varied within the same experiment

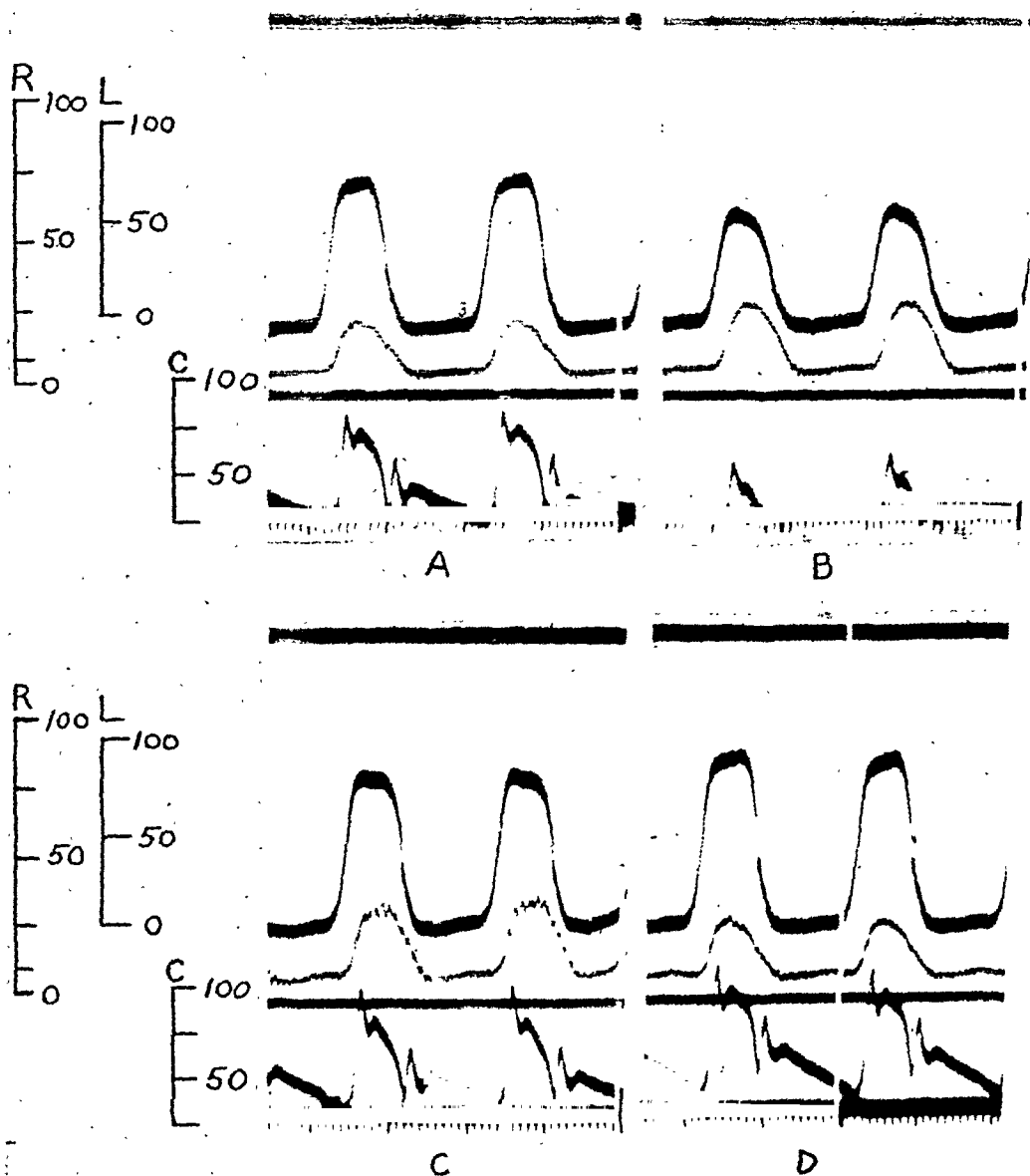


Fig. 2. *A*, PRESSURE PULSES from the left ventricle (upper), right ventricle (middle), and carotid artery (lower) under control conditions. *B*, effects of opening the shunt. *C*, effect of raising systolic discharge by venous infusion with shunt open. *D*, effect of closing shunt. Calibrations on right.

only slightly—not more than .01 second in any case. The time by which the left preceded the right in these six experiments varied from zero to .070 second. In most of the records from two experiments the ventricles contracted simultaneously. Opening the shunt did not alter the respective beginnings of systole in the ventricles appreciably (fig. 4). The chief importance of these observations is that they give assurance that the dynamic alterations of the two ventricles reported were not due to abnormalities of ventricular excitation.

*Changes in contours of right and left ventricular pressure pulses.* As illustrated in figures 2 and 3, opening of the shunt caused the systolic pressure peak in the left ventricle to come earlier in the cycle. From this peak the pressure declined considerably during the remainder of systole. In other words, the pressure is not sustained owing to the decrease in total resistance occasioned by the shunt. The decline of pressure during isometric relaxation also occurred more quickly in the left ventricle when the shunt was open (fig. 4, C—D). Closing this shunt resulted in opposite effects. In the right ventricle opening the shunt caused no decided change in the peak of systolic pressure as indicated in figures 3 and 4 (A—B). However, the summit usually

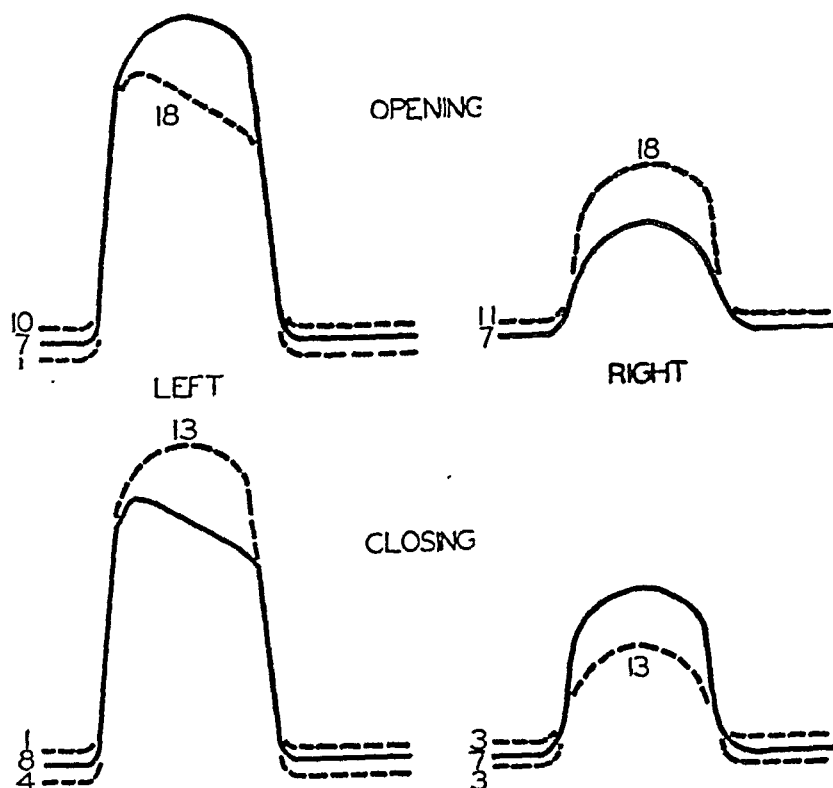


Fig. 3. DIAGRAM summarizing the effects of opening and closing shunt on left and right ventricular pressures. Numerals indicate the number of cases in which the initial pressure changes indicated by dotted lines were found.

broadened. The duration of isometric relaxation was sometimes increased and sometimes decreased (fig. 4, C—D). When the shunt was closed the effects again were more variable on the right side.

*Effective pressure gradient between left and right ventricles on opening the shunt.* It is obvious that the pressure gradient available for shunting blood from the left to the right ventricle must vary during successive moments of systole. The magnitude of these differences was determined by superimposing the right and left ventricular pressure curves brought to common ordinate values by means of Green's co-ordirectograph. In figure 5, graph A shows the relation of the two pressures with the shunt closed. The diagonally shaded area in B indicates the effective pressure differences which eventuated in an actual experiment with the shunt open. As a result of a decline of pressures on the left and an elevation of pressures in the right

ventricle the overall effective pressure difference is much less than normal pressure relations without a shunt lead one to believe. The rôle that a compensatory increase

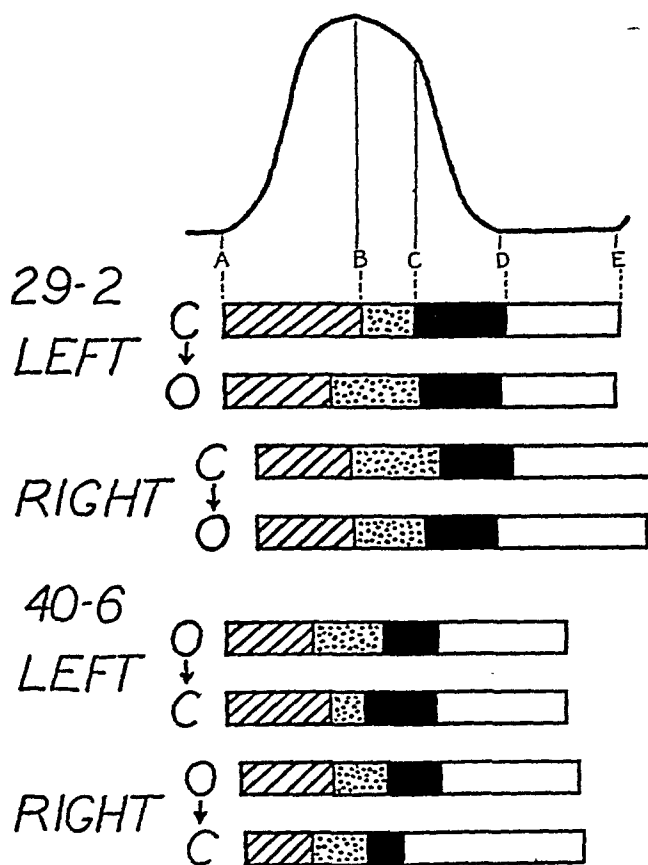


Fig. 4. DIAGRAM illustrating changes in duration of different phases of contraction and relaxation and relative differences in the onset of right and left ventricular contractions. Two experiments are plotted.

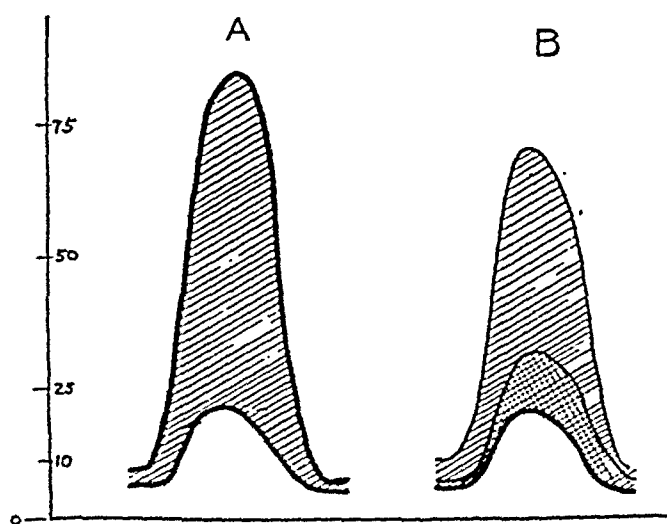


Fig. 5. GRAPHS from actual records indicating pressure differences between the left (upper) and right (lower) ventricular cavities. A, normal pressure differences; B, diagonally shaded area, pressure differences during opening of an interventricular shunt under experimental conditions. Cross-hatched area indicates extent to which physiological responses of right ventricle reduce the differential pressure between the two ventricles.

in right ventricular pressure plays in reducing the pressure differences can be graphically expressed by drawing also a curve of the normal right ventricular pressure. In figure 5B the crossed hatched area represents the effects of right ventricular compensation.

## INTERPRETATIONS

The data presented allow certain deductions as to the manner in which the mechanism of ventricular action is altered by the presence of an interventricular communication. Certain elementary physical facts are obvious. Since the pressure during systole reaches higher levels in the left than in the right ventricle, part of the stroke volume of the left ventricle must be diverted into the right ventricle while less is expelled into the aorta. As a consequence, aortic pressure declines and right ventricular pressure increases during systole.

One question appears never to have been adequately analyzed. Is the higher right ventricular pressure maximum solely due to a summation of pressure energy developed by the right ventricle with that transmitted from the left? Or does the right myocardium create more pressure energy during systole by virtue of certain dynamic changes? The latter may be expected to occur provided the right ventricle is dilated sufficiently to increase the initial length of its fibers. That such a stretch occurred in 11 or 18 experiments is obvious from the fact that initial tension was measurably increased. In 7 experiments however no such change occurred. This does not necessarily signify that no distension took place in the right ventricle during diastole. In a preceding paper (1) evidence was submitted that a considerable increase in volume of the large veins and right atrium can occur before a significant elevation of right atrial pressure supervenes. Similarly, at least in the exposed heart, careful observations and rapid motion pictures have shown (2) that the surface of the filled right ventricle below the origin of the pulmonary conus appears lax, suggesting that some available space exists which can be occupied by additional blood before the fibers are stretched sufficiently to cause a measurable increase in intraventricular tension. It is as though the heart were at first a plastic organ which dilates to accompany the increased volume of blood perhaps with only a minute and unmeasurable increase in initial tension. However, as filling increases more and more the elastic properties come into play with a greater increase in diastolic pressure. For this reason the measurable increase in initial tension found in 11 of our experiments is highly significant and justifies the conclusion that the excess pressure energy developed in the right ventricle is in part at least occasioned by a greater myocardial response.

This compensatory response of the right ventricle serves two useful purposes: 1) It enables the right ventricle to discharge into the pulmonary artery a total volume equal to that which enters from the right atrium during diastole and from the left ventricle during systole. 2) By more nearly equalizing pressures to the right and left of the septum the shunted volume of blood is less than that which would otherwise be transferred according to physical laws. In other words, the magnitude of the shunted volumes depends on the capacity of the right heart to compensate through increase in initial length and tension. Owing to the drastic procedures required in our experiments it is improbable that the compensation was as great as may be anticipated in human developmental defects. Indeed, when the shunt was left open for a long time with the hope that compensatory recovery of arterial pressures would supervene, it was sometimes found that the right ventricle distended more and more while arterial pressures and pulse pressures declined. As a rule a circulatory balance



was slowly restored after closing the shunt. In some of our hearts, however, the compensatory power of the right ventricle had certainly not reached its limit. This is shown by increased response of both ventricles to saline infusion, illustrated in figure 2, B and C. Indeed, this was found to be the most effective method for increasing the effective discharge of blood into the aorta, as judged by restoration of control pulse pressures.

In a condition in which the circulation becomes unbalanced by accumulation of a larger proportion of the total blood volume on the venous side an increase in total circulating volume is apparently an effective mechanism for restoring circulatory balance as long as the right ventricle can respond to the larger input load. The observation that right ventricular volume increased and that this frequently gave rise to an elevation of initial tension supports the theory which holds that hypertrophy so commonly found in human cases is a consequence of the increased distension.

The question may be raised why if the left ventricle empties—perhaps even more effectively than normally—through an opening in the ventricular wall as well as the aorta, the initial pressure should rise on the left side, and perhaps offer a stimulus for the development of hypertrophy on this side as well? The answer is apparently found in the fact that the right ventricle through development of a greater pressure maximum ejects larger stroke volumes into a pulmonary circuit whose resistance has probably not changed materially. This increased output of the right heart is reflected in a greater flow to the left.

The possibility must be considered that under certain conditions the left ventricle in ejecting blood through an interventricular septum does not largely do so at the expense of the blood volume normally expelled into the aorta, but utilizes part of its residual volume. This would explain the failure of initial tension to rise in the left ventricle in 7 of our experiments. Such a postulate must be thrown out of court, however, by the fact that the aortic pulse pressure was immediately reduced and gives evidence of a smaller systolic discharge. The failure of left ventricular initial pressure to rise may possibly be explained by the fact that the walls of the left ventricle are also capable of dilating in a plastic manner before initial pressure is significantly increased.

The normal myocardium adapts itself for the expulsion of large blood volumes by prolonging systole whenever initial pressure or diastolic stretch are increased (3). The increased S/C ratios found in most of our records on opening the shunt gives additional evidence that the natural response of the heart to distention remains unaltered in interventricular septal defects.

#### SUMMARY

The manner in which the heart reacts to an interventricular communication was studied in dogs by use of an external shunt which could be opened and closed alternately. The shunt and its application are described. The constancy of relations between onset of right and left ventricular systole gave evidence that the sequence of ventricular excitation was not disturbed by the apparatus. The dynamic changes, some of a compensatory nature, were deduced from detailed analysis of simultaneously recorded left and right intraventricular pressure curves aided by studies of

aortic pressure pulses. Registrations were made by calibrated optical manometers of adequate efficiency.

The predominant changes in the left ventricle on opening a shunt consisted of an elevation of initial pressure, a decline in maximal pressure and a prolongation of contraction in relation to cycle length, with modification in the contour of pressure pulses. The predominant effects in the right ventricle on opening the shunt consisted in an elevation of initial and maximal pressures.

An analysis of results lead to the conclusion that the great elevation of right ventricular pressure is certainly not caused wholly by summation of right and transmitted left ventricular pressures. It is due in part to a more vigorous response of the right myocardium consequent to increased diastolic distension. This compensatory response acts *a)* to mobilize larger volumes of blood for return to the left heart and *b)* to reduce the fraction of total left ventricular discharge which is transferred to the right ventricle during systole.

When the right ventricle fails to respond to the law of initial length and tension, progressive dilation and rapid circulatory failure supervene. Increase in total circulating volume operates as one of the best compensatory mechanisms through additional right ventricular compensation provided the right ventricle is in good condition.

We wish to thank Dr. David F. Opdyke for his advice and supervision of the experimental work and Dr. C. J. Wiggers for his assistance in the critical evaluation of the data and in editing the manuscript.

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# COMPARISON OF CARDIAC OUTPUT BY A DIRECT METHOD AND THE HAMILTON-REMINGTON PROCEDURE

J. L. DUOMARCO,<sup>1</sup> W. H. DILLON<sup>2</sup> AND C. J. WIGGERS

*From the Department of Physiology, Western Reserve University Medical School*

CLEVELAND, OHIO

**D**URING the course of concurrent cardiodynamic studies it became necessary to evaluate changes in stroke volume and cardiac output within short periods of time. On the basis of theoretical formulations and a few comparisons with other methods for determining cardiac output, it seemed probable that the method of Hamilton and Remington (1, 2) might serve our purpose. Since the validity of their calculations has only been checked against other indirect methods, it seemed important to make such comparisons with a method which measures cardiac output of the left ventricle directly. This seemed especially important in view of the fact that some of the theoretical postulates involved are subject to argument and that their scheme of analysis may not allow sufficiently for changeable factors of the arterial system.

This communication deals with attempts to compare calculated cardiac output by the Hamilton and Remington method with direct measurement of cardiac input.

## METHODS FOR DIRECT AND INDIRECT MEASUREMENT OF CARDIAC OUTPUT

In order to calculate stroke volumes and cardiac output from aortic pressure curves, the latter were recorded in rather large amplitude by modified Gregg manometers. All pressure pulses used were those actually recorded at the same time that direct cardiac input determinations were made. Only records were used in which a stable diastolic pressure was maintained during the entire period. The curves were integrated by the procedures outlined by Hamilton and Remington (1, 2).

Since the validity of comparisons depends on the accuracy with which cardiac input is measured by our direct methods, these require detailed description. Three different procedures were used; or rather data from one procedure already available were used to avoid repetition, and two new techniques were developed.

1. *Comparisons of measured venous inflow into the right heart and calculated left ventricular output.* Four such experiments in which right ventricular input was estimated were available from records obtained by Wiggers, Guevara Rojas *et al.* (3). In these experiments the azygos vein was ligated and blood from the cannulated peripheral ends of the vena cavae was collected in a low level reservoir and pumped to a higher level reservoir from which it flowed through a Ludwig type stromuhr into the

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Received for publication July 19, 1948.

<sup>1</sup> Guggenheim Fellow; present address: Canelones 2013, Montevideo, Uruguay, S. A.

<sup>2</sup> Student Fellow, Life Insurance Medical Research Fund.

right atrium. It is evident that this method does not measure coronary venous flow and that the actual input is thus underestimated, but certainly not by more than 20 per cent.

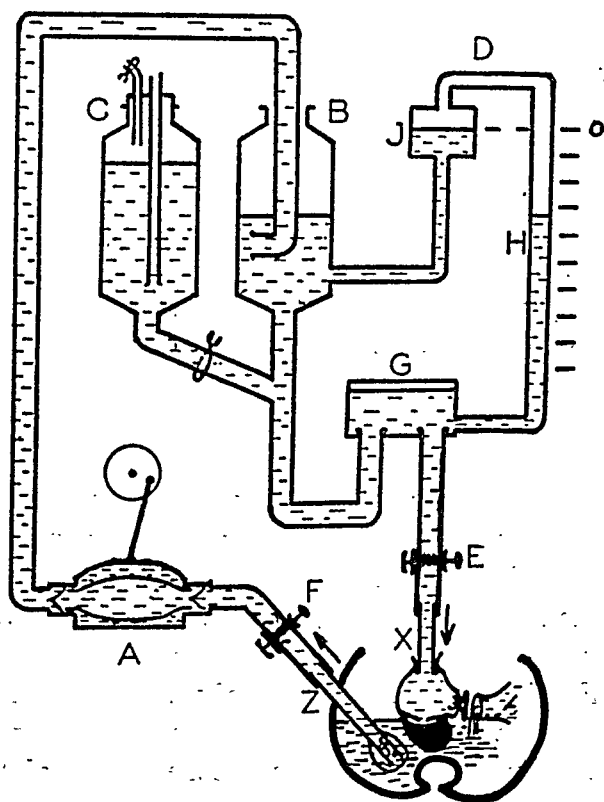


Fig. 1

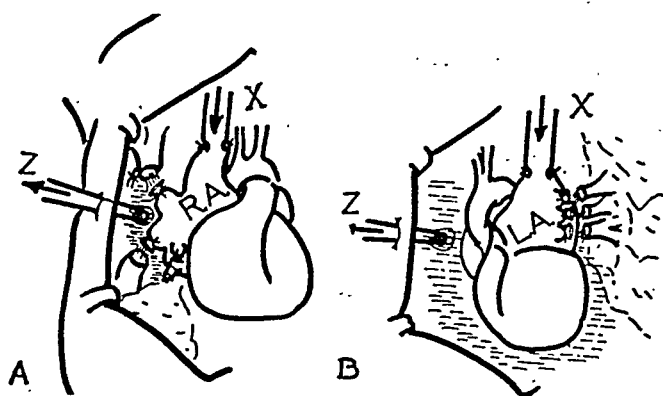


Fig. 2

2. *Comparisons of measured right cardiac input including coronary sinus flow and calculated left ventricular output.* In order to reduce the error, a method for perfusing the right heart was devised by which the coronary sinus flow was included in the measured right ventricular input. It consisted in principle in allowing blood from ligated and severed ends of the inferior and superior cavae and the coronary sinus

to gather in the thoracic cavity. A tube with a tip protected by a wire basket was inserted through an intercostal space so that it rested at the lowest point of the thoracic cavity. Through this tube blood was pumped into the right auricle via a large cannula inserted into its appendage. The apparatus is shown schematically in figure 1. A pump *A* with a small internal capacity and rapid strokes elevated blood to the chamber *B* whence it flowed into an inverted flask *G* which captured bubbles. From this chamber it passed by gravity into the right atrium. The input rate could be controlled by a screw clamp *E*. The rate of auricular inflow is determined by the device *D* which is an adaptation of the venturimeter principle. The difference of manometric levels corresponding to two points of the circuit (taken conveniently from *B* and *G*) can be read in tube *H* because the ampule *J* maintains the other level constant. The apparatus was calibrated after each experiment. In order to check inflow rates, complementary flow rate determinations were made by opening the Mariotte bottle *C* for a given interval of time while bottle *B* was temporarily closed. Aortic pressure curves were taken during the measured inflow period.

A few minor but important details of operation may be indicated. The entire attention of one experimenter is required to keep the circulation in operation. Cardiac input can be graduated at will by stopcock *E* as long as the capacity of the ventricle is not exceeded. Precaution must be taken to keep the tip of the thoracic cannula, *Z*, below the level of blood in the thorax in order to avoid the formation of foam in *B*. This is most conveniently done by graduating the degree of aspiration of blood by clamp *F*. Successful adjustment of clamps *E* and *F* results in a constant level in bottle *B*. The drainage of blood from the veins and the return to the right auricle are illustrated in figure 2A. The detailed steps of the procedure are as follows:

1. The chest is opened and an aortic optical manometer connected.
2. The two upper lobes of the right lung are ablated and the blood drained into the thorax containing heparin solution.
3. The azygos vein is tied and loose ligatures are placed around the two cavae and coronary sinus.
4. A tube with a wire protection at the end is inserted through an intercostal space, and its tip placed at the bottom of the thoracic cavity.
5. The animal is heparinized.
6. A large cannula is inserted into the right auricular appendage.
7. The circuit inside of the animal is filled with heparinized blood from a large donor dog and connected to the auricular and thoracic cannulae.
8. Sufficient heparinized blood is placed in the thorax to cover the thoracic cannula safely.
9. The coronary sinus is tied and the vessel slit allowing blood to flow into the thoracic cavity. The superior and inferior vena cavae are ligated and cut, allowing blood to flow into the chest.
10. The pump is started by another operator, which thereafter continues to supply the heart with blood from the external circuit.

3. *Comparison of measured left cardiac input and calculated output.* This procedure differed from the foregoing in that the left pulmonary veins were tied centrally and cut peripherally, allowing blood after passing the lungs to enter the thoracic cavity. From this it was pumped as before via a left auricular cannula directly into

the left heart, as illustrated in figure 2B. The steps are the same except for the following:

*Step 2.* Loose ligatures are placed around the 4 pulmonary veins of the left side.

*Step 3.* The whole right lung is ablated.

*Step 6.* The left auricular appendage is cannulated.

*Step 9.* As nearly simultaneously as possible the left pulmonary veins are tied and severed, allowing blood to enter the thorax.

The principle difficulty encountered when infusing into the left heart was the occurrence of coronary insufficiency due to air emboli. Minute bubbles which were not removed from the blood in *G* found their way to the coronary arteries and caused the heart to fail in 14 of 17 experiments. The temperature of the blood in the thorax averaged 32°C.

### RESULTS

Comparison of measured right ventricular input and estimated output of the left ventricle was made in 15 records selected from four experiments available from previous work in this department. In these the venous inflow into the right heart minus the coronary flow was measured. In 50 records from four experiments the right cardiac input *including the coronary sinus flow* was compared with calculated left ventricular output. In 16 records from three experiments, comparisons of input into the left heart were made with the estimated output from the left heart.

The individual data of these experiments are incorporated in table 1. Changes in cardiac output in the same animal were usually achieved by altering venous inflow except in the observations followed by an asterisk; in these small doses of neosynephrine were administered. Figure 3 shows in much reduced size 17 of the 81 valid records calculated. The upper 11 records demarcated by a line were from experiments in which the total right ventricular input was measured directly and the lower 6 records exemplify experiments in which left ventricular input was measured directly. On each record are inscribed the calibration, the surface area of the dog (S.A.), the calculated minute output per dog in liters (C.V.), and the directly measured input in liters (M.V.).

A glance at these records shows that while the form of most of the pressure pulses is reasonably good the diastolic pressure was rather low and the pulse pressure large in most animals owing to the artificial conditions of the experiments. However, in six records (20-6; 21-11; 7-13; 7-14; 8-4) the diastolic pressure ranged between 60 mm. and 80 mm. Hg. In all of these instances, except experiment 8-4, the calculated minute output proved to be of the order of about twice the measured ventricular input.

A perusal of the data charted in table 1 indicates that even greater disproportions can occur. This is particularly pronounced in experiments 6 and 7 in which the calculated output of the left ventricle was sometimes four-fold greater than the measured input into the left ventricle. The data also show that while commonly the calculated values show directional trends in output determinations this is by no means always the case when cardiac input is increased or decreased purposely by raising the pressure head for inflow.

TABLE I

RECORD	CALC. OUTPUT, LT. HEART	RT. HEART INPUT, MINUS CORONARY FLOW	RECORD	CALC. OUTPUT, LT. HEART	RT. HEART INPUT, + CORONARY SINUS FLOW
	cc./min.	cc./min.		cc./min.	cc./min.
88-2	1470	1056	19-1A	1643	1875
88-38-39	1260	903	3D	1644	1325
91-10	1480	894	4C	2085	1690
91-11	1105	754	5B	2320	1395
91-16	1100	540	6A	2157	1235
91-20	1760	936	B	1300	790
92-3	2020	984	C	2385	1130
92-7	1710	1280	D	2514	1265
92-8	1510	725	E	2065	1290
92-25	1970	1035	7A	2030	1290
100-6	1340	1091	8A	2295	1410
100-10	1550	672	20-I	2370	995
100-20	1760	299	2	2860	1255
100-37	2294	812	3	3420	1155
100-42	3274	921	4	2830	1200
			5	2370	817
			6	2220	1015
		TOTAL LT. HEART INPUT	8	2030	860
		cc./min.	9	1900	725
6-7	1008	490	10	1780	560
7-4	1550	370	21-I	1710	410
7-5	1630	400	2*	1445	1285
7-6	1980	430	3	2300	1360
7-8	1890	475	4	1310	1185
7-9	2310	505	5	2010	1325
7-10	2350	505	6	1250	1290
7-11*	2300	485	7	2360	690
7-12	1960	690	8	2190	1320
7-13*	1870	685	9	2350	1245
7-14*	2230	675	10	2540	1355
8-1	2290	1090	11	2520	1420
8-2	2690	1570	12	2860	1425
8-3	1560	1380	13	2880	1320
8-4	1140	1325	14	2840	1395
8-5	1670	1400	15	3280	1375
			17	2540	1150
			18	3440	1320
			19	3220	1345
			20	3290	1380
			21	3580	1450
			22	3290	1420
			24	3700	1965
			25	3760	1920
			27	3660	2055
			28	3560	1955
			22-I	1505	770
			2	1582	870
			3*	1990	885
			4	2185	927
			5	2110	850

## DISCUSSION

The astounding differences between measured blood volumes which enter the heart and leave it according to calculated pulse curve measurements clearly indicate

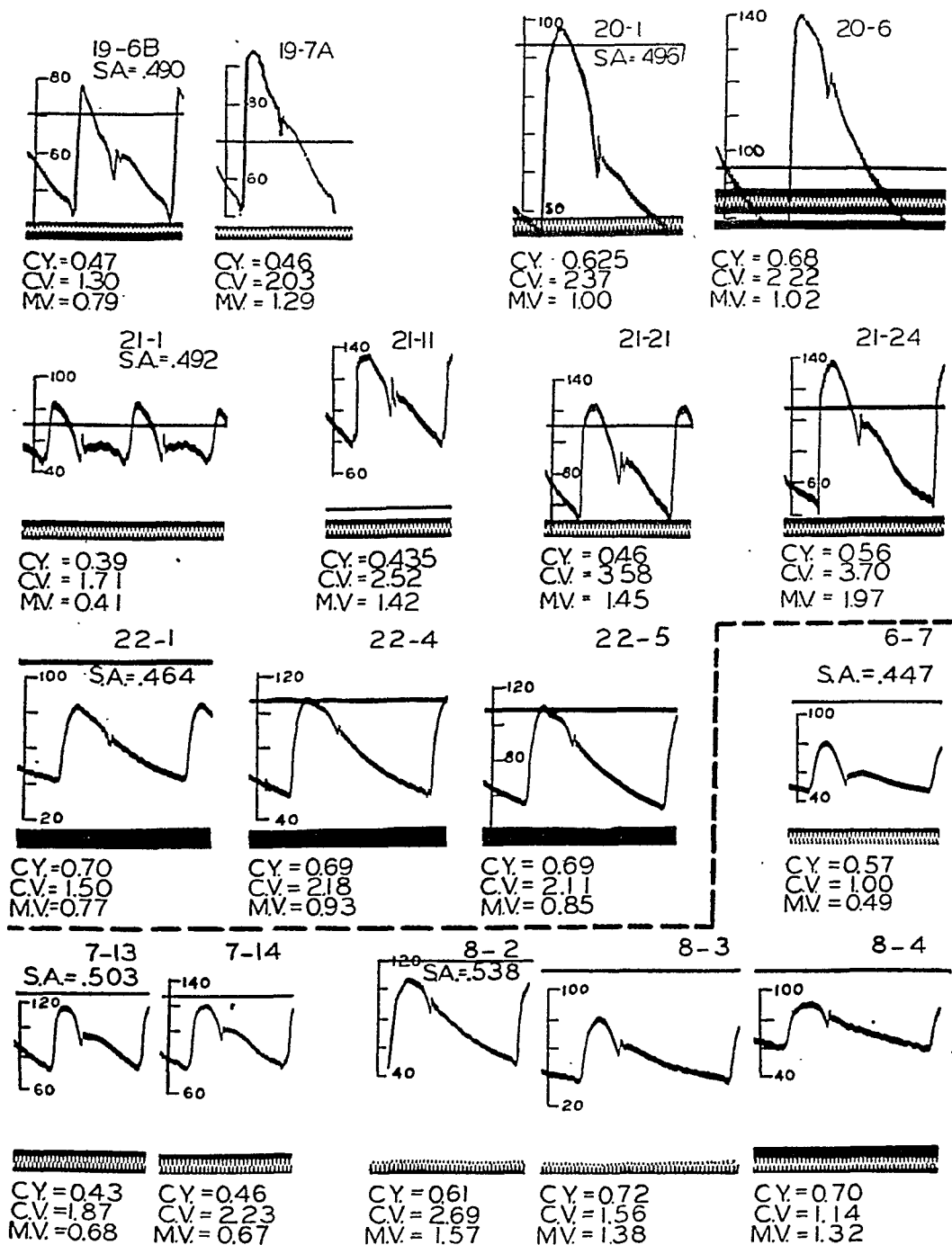


Fig. 3

that the Hamilton-Remington procedure for measuring cardiac output is far from good enough to be used in experimental studies. Some reservations should perhaps be exercised in concluding that equally large discrepancies exist in intact animals



between actual input and output calculated by the Hamilton-Remington method. They strongly suggest, however, that physical principles deduced from determination of averaged constants from dead dogs may not be generally applicable to individual live dogs, and possibly that uncontrollable factors may enter which invalidate their general employment.

It may be asserted with some firmness that discrepancies found in our experiments cannot be waived aside by insinuations that comparisons were made on failing hearts of moribund dogs and that they are therefore inapplicable to the intact circulation. If the postulates advanced by Hamilton and Remington are correct it is only necessary that the left heart eject blood in a reasonably normal manner into an arterial system with normal and unchangeable volume elasticity constants. Indeed, were it not for the coronary system an artificial pump operating in the same manner as the left ventricle could be substituted. No satisfactory artificial pump has so far been designed. The possibility exists of course that the arterial system changed in our experiments, but it seems highly improbable that they deviated from the normal more than in dead dogs from which Hamilton and his associates gathered the data used for generalized calculations for living animals.

#### SUMMARY

Three methods were devised by which the input of the right or left heart per minute could be measured directly, while aortic pressure pulses were recorded in large amplitude by calibrated Gregg manometers. The pressure pulses were analyzed according to the procedure of Hamilton and Remington and cardiac output was calculated.

The analysis of 81 records from 11 dogs revealed that left ventricular output is generally two or more times as large as the measured input; further that in isolated instances the directional trend of cardiac output was not even predictable from such analyses.

While some reservation must be exercised in stating that discrepancies of similar magnitude exist in less extensively operated dogs, the results clearly show that the method is far from good enough to be of use in experimental studies of cardiac output in dogs.

The authors wish to express their appreciation to Dr. David F. Opdyke for advice in the conduct of experiments and to Dr. Robert S. Alexander for suggestions in the analysis of records.

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# EXPERIMENTAL HEMORRHAGIC SHOCK; A STUDY OF ITS PRODUCTION AND TREATMENT

OTTO GLASSER AND IRVINE H. PAGE

*From the Cleveland Clinic and Frank E. Bunts Educational Institute, Research Division*

CLEVELAND, OHIO

THE advances made in the study of shock have not resolved, in conclusive fashion, methods for the consistent experimental production of so-called irreversible hemorrhagic shock (1). The purpose of this investigation was to study the reasons for the inconsistencies which persist and to establish criteria which might lead to better definition of the degrees of shock.

Numerous methods for producing hemorrhagic shock have been described in recent years by Wiggers and Werle (2, 3), Wiggers, Ingraham and Dille (4, 5), Kohlstaedt and Page (6, 7), Glasser and Page (18), Seligman, Frank and Fine (8, 9), Walcott (10), Pasqualini (11), Sayers, Sayers and Long (12).

The principle of the procedure adopted in most of our experiments was described by C. J. Wiggers and Werle in 1942 (2, 3). Under general anesthesia, a dog is bled rapidly from a femoral artery into a reservoir containing heparin solution, until mean arterial pressure is indicated as 50 mm. Hg by a mercury manometer. This *moderate* hypotension is sustained for 90 minutes and is followed by a period of *drastic* hypotension at a mean arterial pressure of 30 mm. Hg lasting 45 minutes so that the total hypotensive period is 135 minutes. In their procedure, all the blood is then reinfused intravenously.

In our experiments two modifications of this technic were introduced. One is the maintenance of a permanently open connection between animal and recording reservoir under the desired positive pressure throughout the experiment. The other involves entirely replacing intravenous by intra-arterial transfusion of the removed blood (6, 7, 13, 14).

Cardiac intra-arterial transfusion has elicited little interest. The principle of the method was described as early as 1875 by Landois (15). In crude form, it was used successfully in an occasional patient by Halsted (16) in 1883 and by a few other surgeons (Huetner, Crile). A number of difficulties occurred subsequently and the method fell into disuse. Its revival was suggested to us by Colonel Sam Seeley, M.C., U. S. Army.

## METHOD OF PRODUCING HEMORRHAGIC SHOCK

Dogs, weighing from 4.5 to 20.7 kg. and averaging 10.7 kg., were fasted for 24 hours and then anesthetized by subcutaneous injection of 5 mg/kg. of morphine sulphate and intraperitoneal injection of 30 mg/kg. sodium pentobarbital.<sup>1</sup>

A glass cannula (A, fig. 1) was inserted into a femoral artery, and connected through one branch of rubber tubing with a mercury manometer (B) recording arterial pressure on a slow-moving kymograph. The other branch lead to a recording

Received for publication June 25, 1948.

<sup>1</sup> Several experiments carried out under local anesthesia (2% solution of novocain) produced results which were similar to those obtained with general anesthesia. In some of these experiments, sudden movements could not be avoided, and curare (intocostrin, 20 U per ml.) was given intravenously in doses of 0.2 ml. at intervals of 5 minutes up to a total of 1.6 ml. Administration of curare in combination with severe hemorrhage led rapidly to serious respiratory difficulties and was therefore abandoned.

reservoir (C) in which the withdrawn blood was stored. A small side arm of this line was equipped with a stopcock adapter to which a Luer syringe could be attached for the purpose of injecting anticoagulant into the tubing.

The reservoir (C) was suspended on a balance spring and equipped with a pen to record changes in its weight on the kymograph. This allowed observation of the movements of blood back and forth from the animal to the reservoir during the course of the experiment. The stopper of the reservoir held two tubes; one short, through which the blood flowed and a long one through which air escaped or through which air could be pumped by a small bulb (D) to control blood movement between reservoir and animal. Pressure within the reservoir could be read on a mercury manometer (E) or a sphygmomanometer (F). An air outlet with adjustable valve

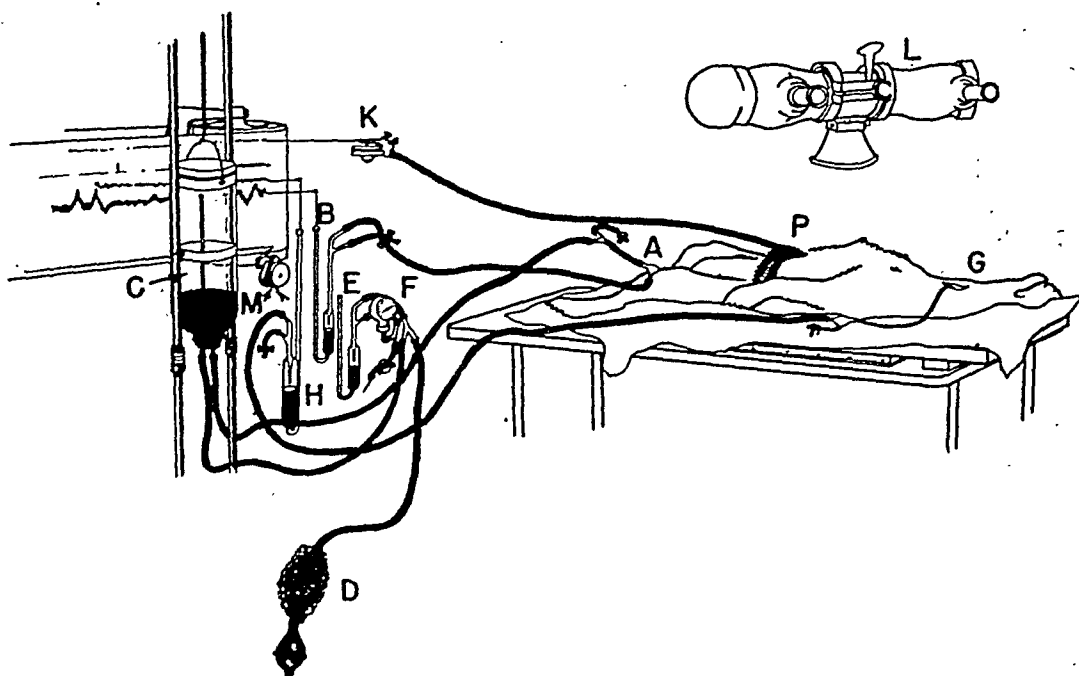


Fig. 1. APPARATUS FOR BLEEDING and arterial transfusion (see text)

was inserted in this line near the sphygmomanometer. This and the bulb (D) permitted complete control of air pressure in the reservoir.

A no. 12 French rubber catheter was inserted into the external jugular vein (G) with the tip approximately at the level of the atrium, and connected through a glass cannula to a water manometer (H) to record venous pressure on the kymograph. Respiratory rate was recorded (K) from a pneumograph (P). Time intervals of one minute obtained from a synchronous clock motor (M) were recorded on the base line.

Response of arterial pressure to a dose of 0.1 to 0.2 ml. of a 1:10,000 adrenalin solution was regularly ascertained before bleeding during hypotension, after transfusion. Rectal temperature, room temperature, relative humidity and barometric pressure were recorded for each experiment. Electrocardiograms and electroencephalograms, hematocrit and hemoglobin values before, during and after hypotension, and radiographic examination of heart were made in a number of experiments (x-ray tube L).

## PROCEDURE

Before the beginning of the experiment, all connections from the cannula in the femoral artery and catheter in the jugular vein to manometers and reservoir were sterilized and filled with heparin-saline solution (100 mg. heparin per 1000 ml. 1% saline).

Bleeding was started by releasing the clamp which closed off the line between cannula (A) and bottle (C). With the air outlet open, the blood entered the reservoir. Bleeding was done rapidly, at an average rate of 100 ml/min. When the mean arterial pressure reached 50 mm. Hg, the air outlet was closed. By manipulation of bulb and air outlet, the pressure was held at 50 mm. Hg for 90 minutes. During the period in which the blood flows from animal to reservoir, danger of clotting exists; consequently, 5 ml. of heparin-saline solution was injected from time to time into the side arm near the cannula (A). While injecting this solution, both connections from cannula (A) to manometer (B) and bottle-reservoir (C) were clamped off. If heparin solution was to be injected into the reservoir (C) alone or if the blood in the reservoir was to be 'washed' or thoroughly mixed with this solution, the short-connection between cannula (A) and fork with side arm was clamped off.

After the 90-minute period of *moderate* hypotension at 50 mm. Hg, the pressure inside the reservoir was lowered by opening the air outlet near the sphygmomanometer (F). Further bleeding took place although the amount of blood required to drop the arterial pressure to 30 mm. Hg was small, averaging only 7 per cent of the total amount removed. The period of *drastic* hypotension at 30 mm. Hg was maintained for at least 45 minutes, after which all or part of the blood stored was reinfused into the same femoral artery, by increasing the air pressure in the reservoir. The average rate of infusion was 130 ml/min. Infusion was usually stopped when the mean arterial blood pressure reached about 100 mm. Hg.

Early in the investigation, between July 1945 and June 1946, 31 animals were submitted to this procedure to determine its effect on survival. Experimental data are presented in table 1.

Since the same system of numbering the columns is used in all tables, the following describes a typical table.

Experiment number is given in Column I, the amount of blood removed in ml/100 gm. body weight in Column II, time of hypotension in minutes in Column III. Column IV shows relative intake of blood by the animal during the hypotensive period, whereby 0 signifies no intake, x slight, xx moderate, xxx considerable and xxxx large intakes. Intake into the animal during transfusion to reestablish arterial pressure at the level of 100 mm. Hg in per cent of total volume of removed blood is given in Column V; Column VI shows the pressor response to adrenalin after reinfusion in per cent of the response to the same dose before bleeding. Column VII gives the survival rate of animals after the shock procedure; permanent survival is indicated by 'yes', temporary survival by the number of hours up to 36 and death within one hour after transfusion by 'o'. In each column the animals are divided into two groups, I and II, according to prognostic criteria which will be discussed below.

The average duration of hypotension for the experiments in table 1 was 143 minutes and the average volume of blood removed to produce shock was 5.2 ml/100

TABLE 1. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED ONLY WITH INTRA-ARTERIAL TRANSFUSION (EARLY CONTROL EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
22		4.6		135		o		27				Yes
23		5.5		150		o		37				27
25		4.8		142		o		47				2
26		2.6		135		o		64				10
43	5.5		139		xxx		69				o	
71	5.7		156		xx		100				o	
72	4.4		156		xx		100		80		Yes	
84		6.1		150		o		20		85		10
85	5.5		135		xxx		100				o	
100	5.2		135		xxxx		100				o	
102		4.7		150		x		39		100		Yes
103	4.9		135		xxxx		100				o	
105	6.0		141		xx		63		80		10	
105		4.6		144		x		29		100		Yes
107	4.1		135		xx		50				o	
108		3.5		136		x		60		80		Yes
111		4.2		150		x		31		66		10
112		6.1		137		x		33		100		5
113		4.4		152		x		31		40		14
115	6.5		141		x		79				3	
116	7.2		146		xx		79		30		3	
117		4.9		135		o		50				5
121		5.6		139		x		46				3
122	4.8		137		xx		50		50		5	
139	5.3		136		xxx		92				5	
140	5.2		135		xx		100		40		5	
141		7.8		159		x		42		40		10
142		5.6		158		o		100		75		Yes
143		5.2		147		o		50				10
167		5.9		139		x		40		50		Yes
187	4.3		136		xxxx		100				o	

Number of experiments in Groups I and II:	Group I: 14	Group II: 17	Total: 31
Survival, permanent:	1 ( 7%)	6 (35%)	7 (23%)
Survival, 1 to 36 hours:	6 (43%)	11 (65%)	17 (54%)
Survival, less than 1 hour:	7 (50%)	0	7 (23%)

gm. body weight. Of the 31 animals, 23 per cent survived permanently after transfusion and 54 per cent died within one to 36 hours (average 8 hours) while 23 per cent died within less than one hour.

The early series of 31 control animals was followed by a later series of 29 control

animals which were submitted to the shock procedure between September 1946 and January 1948. The experimental data for this group are presented in table 2.

Average duration of hypotension for these animals was 150 minutes and average

TABLE 2. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED ONLY WITH INTRA-ARTERIAL TRANSFUSION (LATE CONTROL EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL. ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
228		2.7		141		X		100				Yes ✓
229		5.4		135		X		100		91	Yes	10
373	4.6		137		XXX		100		55		Yes	
375		4.8		137		o		50		81	Yes ✓	
376		3.7		135		o		40		100	Yes ✓	
377	3.6		141		XX		100		23		4	
378		6.6		141		o		46		100	Yes	
381		6.3		142		o		89		86	Yes	
382		5.6		137		o		55		53	Yes	
383		4.1		151		X		67		100	Yes	
384		4.1		145		X		67		33	Yes	
385		5.0		167		o		67		37	Yes	
386		5.3		150		o		53		65	Yes	
387		5.3		157		o		57		35	Yes	
388		4.3		153		o		75		56	Yes	
389	4.6		143		XX		33		31		6	
390		6.1		159		o		56		100	Yes	
391		8.2		154		o		88		75	Yes	
392		4.3		142		o		66			Yes	
395		4.9		156		XX		62		85	Yes	
397		5.1		142		o		65		100	Yes	
415	3.7		142		XX		100		30		5	
416		4.7		142		X		84		80	15	
417		4.5		227		X		100		60	5	
421		4.6		142		X		78		134	Yes	
422		4.8		165		o		82		83	Yes	
434		4.5		163		X		43		43	6	
435		4.0		147		X		70		50	Yes	
436		5.5		150		XX		56		36	Yes	

Number of experiments in Groups I and II:

Group I: 4	Group II: 25	Total: 29
Survival, permanent: 1 (25%)	21 (84%)	22 (76%)
Survival, 1 to 36 hours: 3 (75%)	4 (16%)	7 (24%)
Survival, less than 1 hour: 0	0	0

amount of blood removed was 4.9 ml/100 gm. body weight. Of the 29 dogs, 76 per cent survived permanently and 24 per cent lived from 4 to 15 hours with an average of 7 hours. None of the animals lived less than 4 hours after transfusion.

Despite the fact that there was wide difference in individual animals in their



liable index to prognosis but taken together they yield reasonably accurate predictions.

We shall call *Group I* those animals in which, according to these criteria, the prognosis is bad, and *Group II* those in which it is good. In the 31 early control animals of table 1 in which no other treatment than arterial transfusion was employed, 14 fell into *Group I* and 17 into *Group II*. Only 7 per cent of *Group I* survived indefinitely, 43 per cent lived an average of 4 hours and 50 per cent died shortly after transfusion. On the contrary, of *Group II*, those with the criteria of good prognosis, 35 per cent lived indefinitely, 65 per cent lived an average of 10 hours and

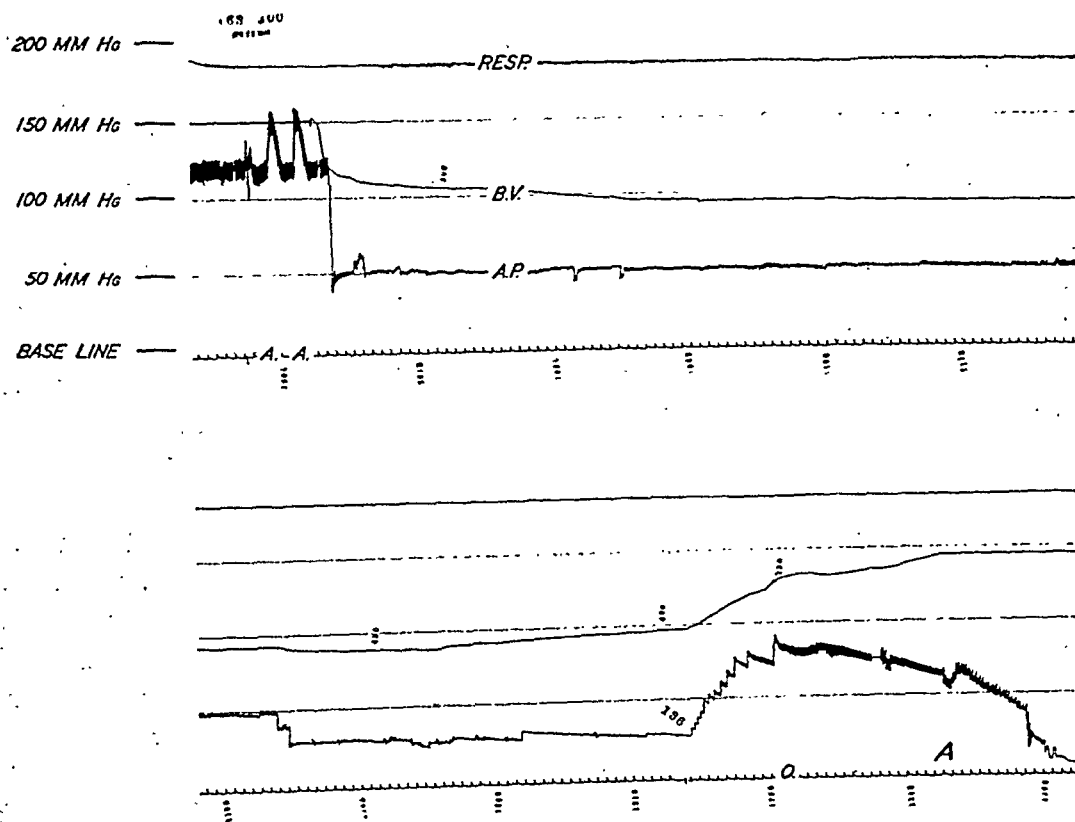


Fig. 3. RECORD OF DOG with bad prognosis after hemorrhage. Symbols as in fig. 2. Note intake of blood during the latter part of the hypotensive period, large intake to restore blood pressure to control level, poor response to adrenalin.

none died within less than 2 hours. Of the later series of control experiments (table 2) *Group I* comprised only 4 animals and *Group II*, 25 animals. One of the 4 animals in *Group I* and 21 (84 per cent) of the 25 animals in *Group II* survived permanently. The remainder lived from 4 to 10 hours and none died within less than 4 hours. Thus on the rigid basis of survival rates alone, the criteria indicate their usefulness.

Bleeding volume in all our shock experiments is given in terms of ml/100 gm. of body weight. The average amount of blood removed in all the experiments described in this paper was 5.4 ml. per 100 gm. body weight. Recalculation of blood removed on the basis of percentage of surface area instead of body weight did not yield a better correlation with the degree of shock or with survival rates. If the calculated bleeding



volume in percentage of body surface is taken as the basis for the amount of blood withdrawn, a relatively greater amount of blood was taken from large animals than from smaller ones. Using the formula  $A = 11 \times W^{0.67}$ , where  $A$  is the surface area in  $\text{cm}^2$ , and  $W$  the body weight in gm., the surface areas for a 10-kg. dog and a 5-kg. dog are found to be  $5265 \text{ cm}^2$  and  $3309 \text{ cm}^2$ , respectively. Thus, the mean bleeding volume of 5.4 per cent of body weight means that 540 ml. of blood was withdrawn in the large dog and 270 ml. in the small dog. Thus  $10.2 \text{ ml}/100 \text{ cm}^2$  surface area were taken from the large dog and  $8.2 \text{ ml}/100 \text{ cm}^2$  from the small dog. Taking bleeding volumes per unit surface area as standard, our larger animals were therefore bled more severely than the smaller ones, although the difference of 25 per cent as indicated in the extreme example cannot be considered as decisive. Still the larger animals should have shown more severe shock and therefore lower survival rates. However, the percentage survivals for shock experiments are practically the same for both groups.

This was also borne out by an analysis of resuscitation experiments which will be presented later in this paper (table 6). Of 13 permanent survivors of that series the average weight was 9.4 kg. and the bleeding volume  $6.0 \text{ ml}/100 \text{ gm. body weight}$ . For 26 nonsurvivors, the average weight was 10.2 kg. and the bleeding volume  $5.8 \text{ ml}/100 \text{ gm. body weight}$ . The surface areas of the two groups are  $5055 \text{ cm}^2$  and  $5336 \text{ cm}^2$ , respectively, which makes the bleeding volumes in percentage of surface area 11.2 ml. and 11.1 ml./ $100 \text{ cm}^2$  surface area. This again indicates that there exists no definite correlation between survival and bleeding volumes measured either in percentage of body weight or body surface.

Although the averages of both bleeding volumes and duration of hypotension are rather uniform for the various groups of experimental animals, individual variations are considerable. While about 13 per cent of the animals used were unable to complete the prescribed period of 135 minutes of hypotension, there were several which withstood the hypotensive period for a much longer time. Thus, about 25 per cent of the animals which withstood more than 135 minutes of hypotension averaged 166 minutes with an extreme survivor of almost 4 hours. This extraordinary ability to withstand severe hypotension and still survive without obvious residual defects is also illustrated by one experiment in which after rapid bleeding the pressure was kept between 30 and 35 mm. Hg for 142 minutes. Following arterial transfusion the dog survived indefinitely, apparently uninjured. These observations agree with those of Kohlstaedt and Page (6, 7) in demonstrating the remarkable inherent ability of some animals to survive severe hemorrhage far more effectively than others. Thus to ascertain the effect of supposed therapeutic procedures, the investigator is faced with either using a larger number of animals, so securing a statistically valid result, or, alternatively, using objective criteria indicating the response of the body to hemorrhage. Both objectives were striven for in our studies. While the total number of dogs used in this investigation with all its ramifications was almost 500, we present in this paper only observations on 244 animals, all of which underwent a hypotensive period of at least 135 minutes.

Division of shocked animals into *Groups I* and *II* according to the prognostic signs described above proved of great value, since attempts to lump animals with

such manifestly different responses to the same experimental conditions into one group would easily produce misleading results.

We indicated several years ago that the response to adrenalin and angiotensin and the rate at which the heart dilated gave some indication of prognosis. In the past four years, measurement of the exchange of blood from a reservoir, in which there is a fixed pressure, with the arterial circulation has been added, along with the requirement of blood to refill the vascular tree at a normal level of arterial pressure. Essentially, these criteria seem to measure the ability of the animal to maintain a certain vascular capacity and to respond to changes in capacity which, in our opinion, may in a measure be reflected in the responsiveness of blood vessels to humoral stimuli. If this interpretation is correct, it is apparent that the inability to prevent arterial pressure from persistently falling below 30 mm. Hg during the hypotensive period indicates a tendency either to loss of peripheral resistance or to weakening of the heart, both of which occur in the terminal phase of shock. They occur early in some animals and it is these that we place in *Group I* as having had prognosis.

While practically none of the animals with unfavorable prognosis survived the shock procedure, the fate of the dogs with favorable prognosis was not as clearly predictable. This is illustrated by a comparison of the survival rates for the early and the late control series as presented in tables 1 and 2. Although all of the experiments were performed by the same group of experimenters, there is a marked increase of permanent survivors in the control series in the later two years of the investigation, 84 per cent as compared to 35 per cent in the initial year of the work for *Group II* animals. Some factors which have contributed to this improvement are improvement in the skill with which the operations are carried out and better aftercare of the animals. Blood clotting, which frequently interfered in the earliest experiments, was eliminated. Attempts to reinfuse quickly the full amount of blood which had been removed after the hypotensive period led in early experiments to irregular cardiac action and, when the overtransfusion was continued, occasionally to heart failure. The amount of blood reinfused was therefore reduced to an average of 70 per cent of that withdrawn. In addition to these obvious causes for increase in the survival rate in the course of our experiments, other obscure factors exerted possibly an even greater influence. This is demonstrated to some extent in the diagram, figure 2, which represents a correlation between permanent survival rate and date of experiment for 244 animals.

The dots in figure 2 represent the percentage of permanent survivors for three week periods from June 1945 to April 1948. For the early period a continuous increase in the survival rates is evident. However, from July 1945 to the present there suddenly occurred periods extending from a week to several months during which prognostic criteria for most animals turned unfavorable. Such an occurrence was as low percentage average to low survival rates as would be expected for *Group I* animals in May 1946, May 1947 and November 1947. The longest low survival period, however, extended practically over three months from February to May 1948 and occurred after experience had been gained in experiments with regard to shock. During this time there were several two week intervals with a survival rate of 100 per cent based on 5 shocked control animals during the week starting May 17, 1948, and of course

this picture suddenly; all 5 control animals were given a good prognosis and they survived permanently. Various suggestions have been made to explain the sudden periods of low resistance to the shock procedure; these include illness of the animals, bacterial infection, seasonal effects, climatic conditions and sudden change of food and environment. We have been unable to correlate any of them with these changes.

It may be of interest to report here that the mean arterial pressure of 244 animals under pentobarbital anesthesia before bleeding was 130 mm. Hg with extremes of 70 and 185 mm. Hg. Mean arterial pressure one hour after transfusion was 98 mm. Hg with extremes of 60 and 150 mm. Hg for permanent survivors.

Although a variety of agents were tried during our experiments, only those which appeared to have a significant effect upon the shocked animal will be described. Ouabain was first used because Kohlstaedt and Page (7) had observed that cardiac dilatation after prolonged hypotension was an important sign of impending terminal shock and when it appeared treatment by blood transfusion alone failed. We have confirmed these findings and proceeded to investigate whether ouabain had beneficial

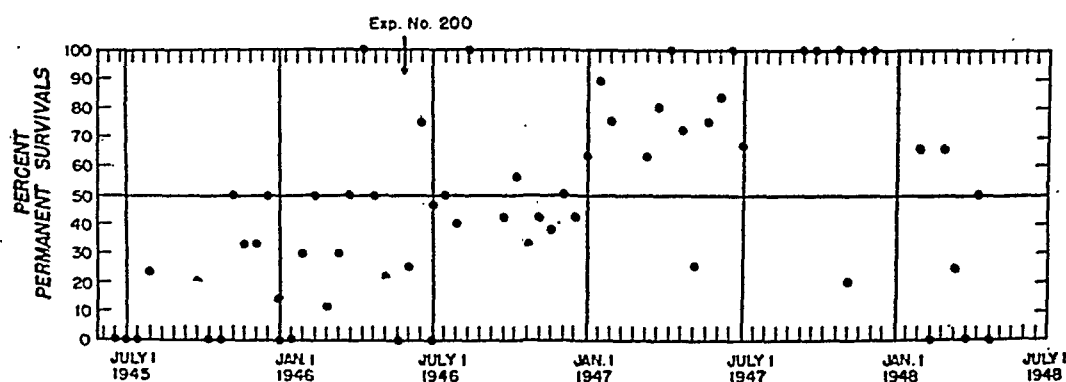


Fig. 4. PERCENTAGE PERMANENT SURVIVAL for 2-week periods from July 1945 to June 1948. No division is made between those with good or bad prognosis.

effects on the heart when given either during or shortly after arterial transfusion. Sixty-one animals were submitted to the shock procedure early in the course of our investigation (approximately throughout the same period of time as the control animals in table 1) and were given 0.05 mg/kg. body weight of ouabain intravenously during or shortly after the transfusion (table 3).

Twenty-two dogs had unfavorable and 39 favorable prognoses. Despite ouabain, all 22 of the former group died within 24 hours, the majority living 5 hours. In contrast 74 per cent of the 39 of the latter group survived indefinitely and 26 per cent lived an average of 7 hours. This contrasts with the control group in which 35 per cent survived while 65 per cent lived an average of 10 hours.

Later in the course of the experiments (table 4) 33 more animals were studied. Only 3 of these had poor prognosis and 30 good. Despite ouabain none of the former and 83 per cent of the latter survived permanently with 17 per cent surviving an average of 14 hours. This compares with 84 per cent survival and 16 per cent in the later control group.

Thus ouabain seemed to increase survival only in the early experiments in which prognosis was good. In this early group there was a tendency to overtransfuse by

TABLE 3. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION AND OUABAIN (EARLY OUABAIN EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
27		3.6		135		o		100				Yes
76	5.0		135		xxx		75		7		23	
77		6.0		180		x		60		93		Yes
78		5.6		172		o		32		100		Yes
79		5.6		152		x		52		46		Yes
80		4.3		153		x		34		19		3
82		6.7		180		x		24		40		10
83		4.4		180		o		36		100		Yes
86	4.8		150		xxx		80				1	
87		7.1		160		xx		50				10
88	5.1		150		xxx		50		12		1	
89		5.0		145		x		19		88		10
90	5.1		137		xx		39			63	10	
91		6.1		157		o		18				Yes
92	5.6		138		xxx		56		33		7	
93		5.0		153		x		16		100		Yes
94		5.8		167		o		36		70		Yes
95	5.5		137		xxxx		100		19		2	
97	5.2		143		xxx		43		30		2	
98	4.7		141		xxxx		36		25		2	
99	5.8		142		o		55		50		5	
101		4.4		137		x		25		57		Yes
118		7.8		137		x		32		50		Yes
119		5.0		140		x		36		85		7
123	5.2		135		xxx		83		60		7	
124	5.0		137		xx		62				0	
126		4.4		137		x		100		100		Yes
131	6.8		137		xx		50				3	
132		5.6		137		o		55		80		Yes
133		5.8		144		x		76		80		Yes
134		5.8		147		o		50		40		Yes
135		6.6		147		o		28				Yes
136	5.6		142		xx		53		50		6	
138		6.0		152		x		54		40		Yes
144		4.9		164		x		47				Yes
146		5.6		143		o		27		40		Yes
147		7.1		136		xxx		47		40		Yes
148		6.0		143		o		40		45		Yes
149		6.3		144		o		29		50		Yes
150	5.9		143		xxx		65				1	
154		4.4		149		o		37		65		Yes
155		4.2		135		o		73		100		Yes
156		6.0		163		x		42		100		6
157		6.4		139		x		50		60		Yes
158		7.3		147		xx		33		20		6
159		6.8		183		o		27		35		Yes

TABLE 3.—Continued

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
160		6.3		139		X		30		35		3
161		5.8		140		XX		25		64		6
162		6.8		136		X		28		100		Yes
163	4.6		137		XXX		60				0	
164	4.9		139		X		79		50		2	
173	6.5		135		XXX		50		10		6	
178	3.0		135		X		100		5		6	
179	6.6		136		XXX		30		50		3	
181	3.9		137		XXX		50		50		3	
182		4.5		136		0		15		10		Yes
183	3.5		138		XX		30				2	
184		5.6		136		0		45		40		11
185		4.1		137		0		45		45		Yes
186	5.1		137		XXXX		100				1	
196		4.2		135		0		50		70		Yes

Number of experiments in Groups I and II:

	Group I: 22	Group II: 39	Total: 61
Survival, permanent:	0	29 (74%)	29 (47%)
Survival, 1 to 36 hours:	20 (91%)	10 (26%)	30 (50%)
Survival, less than 1 hour:	2 (9%)	0	2 (3%)

giving back all of the blood removed. Whether this was in part counteracted by the ouabain we can only guess.

The tetraethyl ammonium ion, following the early work of Burn and Dale (20), has recently been proved by Acheson and Moe (21) to block transmission of impulses over autonomic ganglia.

Ten years ago, Freeman *et al.* (23) published an important paper in which they demonstrated that after total sympathectomy, blood pressure could be reduced to lower levels and for longer times without producing shock than in normal animals. But they were unable to tolerate as large hemorrhages. The difference in reaction was correlated with the peripheral blood flow. In normal dogs, as the blood pressure was reduced by hemorrhage to 70 mm. Hg, blood flow was reduced below 2 ml. per minute, while in the sympathectomized animal it was above 2 ml. The preferential treatment of blood supply to vital centers is lost in sympathectomized animals, but as long as these centers receive sufficient blood supply, all the tissues of the body probably receive an adequate amount of blood and shock is prevented. H. C. Wiggers *et al.* (22) also have demonstrated a deleterious effect of sympathetic over-activity by blocking with dibenamine. This appeared to delay the onset of terminal shock. For these reasons, it was of particular interest to determine the effect on the response to hemorrhage of blockade of all autonomic ganglia.

The animals, 51 in number, were subjected to the shock procedure and either before or within one hour of bleeding given 10 mg/kg. body weight of tetraethyl

ammonium chloride intravenously. A sharp fall in blood pressure occurred, especially when it was given before bleeding. But even after bleeding, the injection

TABLE 4. SURVIVAL RATE AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION AND OUABAIN (LATE OUABAIN EXPERIMENTS)

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
221		3.4		135		x		90				Yes
223		3.8		135		x		100		100		30
230		6.2		135		x		38				11
232		9.3		135		x		100		50		Yes
234		4.7		161		o		100		80		Yes
236		5.4		139		x		77		80		Yes
238		4.8		143		o		37		100		Yes
239	3.1		148		xxx		100		5		10	
240	6.1		273		xxx		83		5		10	
241		4.8		145		x		50		87		Yes
243	4.4		135		xxx		50		0		10	
258		5.7		137		x		100		70		Yes
270		4.8		137		o		75		75		Yes
292		6.3		143		x		62		37		10
295		5.0		143		x		70		30		Yes
296		5.2		143		o		38		56		Yes
297		7.6		163		o		32		60		Yes
298		6.4		149		o		50		108		Yes
299		6.5		180		x		50		37		Yes
300		6.2		153		o		36		154		Yes
302		6.2		159		o		45		100		Yes
305		5.3		185		x		100		57		Yes
306		6.4		144		x		32		50		Yes
308		5.4		162		o		65		80		10
309		5.5		160		x		50		12		10
310		6.3		147		o		90		160		Yes
311		7.6		145		o		44		42		Yes
312		5.8		139		o		55		42		Yes
313		6.2		145		o		36		37		Yes
418		5.9		144		x		94		83		Yes
423		4.8		149		x		100		50		Yes
424		6.3		180		o		39		60		Yes
429		5.0		174		x		72		83		Yes

Number of experiments in Groups I and II:

	Group I: 3	Group II: 30	Total: 33
Survival, permanent:	0	25 (83%)	25 (76%)
Survival, 1 to 36 hours:	3 (100%)	5 (17%)	8 (24%)
Survival, less than 1 hour:	0	0	0

elicited a considerable fall in arterial pressure which was compensated for by the influx of about 23 per cent of the total amount of withdrawn blood over periods averaging 16 minutes. The response to intravenously injected adrenalin was greatly

augmented by the tetraethyl ammonium as Page and Taylor (25) had shown. Re-transfusion was always followed by an injection of ouabain. Survival data are presented in table 5.

Almost all of the animals, 23 out of the 24 with favorable prognosis, survived.

TABLE 5. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK IN ANIMALS TREATED WITH INTRA-ARTERIAL TRANSFUSION, OUABAIN AND TETRAETHYL AMMONIUM CHLORIDE

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BLEEDING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
323		5.5		145		o		90		150		Yes
325		7.0		141		x		100		112		Yes
327		6.7		145		o		71		270		Yes
328		6.2		172		x		47		50		Yes
329	6.6		158		x		100				10	
330		6.6		165		x		66		100		Yes
331		5.7		143		x		71		72		Yes
332	5.0		143		xx		69		120		Yes	
336		6.0		160		o		62		96		Yes
338		5.2		162		o		68		50		Yes
339		4.3		156		x		75		178		Yes
340		5.8		142		x		46		55		Yes
341		5.3		261		x		48		110		Yes
342	5.1		145		xx		83		51		Yes	
344	6.0		158		xx		75		94		Yes	
345		4.8		181		x		62		48		10
346		5.4		151		x		48		53		Yes
347	6.5		141		xx		44		50		Yes	
348	5.6		140		xx		100		100		10	
349		7.7		148		x		100		133		Yes
350		5.6		150		o		50		41		Yes
351	4.0		144		xx		84		150		Yes	
352	6.3		140		xx		44		124		Yes	
353		5.9		160		o		41		100		Yes
354		7.4		164		o		64		134		Yes
356	5.6		149		xx		46		80		Yes	
357		5.1		139		o		53		83		Yes
358	4.6		152		xx		63		73		Yes	
359		4.4		171		x		87		145		Yes
360		5.0		152		x		38		80		Yes
369	4.8		141		xx		40		55		10	
370	4.0		139		xx		34		45		10	
425		4.8		140		x		90		81		Yes
426		8.4		204		o		77		67		Yes
427		5.8		238		o		75		210		Yes
428		3.4		172		x		67		75		Yes
440	3.8		139		x		83		100		15	
441	3.7		142		xx		100		100		8	
442	5.9		153		x		30		100		Yes	
443	3.6		145		x		96		60		6	

TABLE 5.—*Continued*

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. VT.		III TIME OF HYPOTENSION, MIN.		IV REL. INTAKE DURING HYPOTEN.		V INTAKE DURING TRANSF., % OF BULGING VOL.		VI ADRENALIN RESP., % OF ORIG. RESP.		VII SURVIVAL	
	I	II	I	II	I	II	I	II	I	II	I	II
444	5.3		149		X		70		88		8	
445	5.1		147		XX		71		100		Yes	
448	6.0		135		XX		44		43		15	
449	5.4		141		XX		33		90		Yes	
450	2.8		145		XX		64		100		8	
451	4.1		139		XX		38		38		12	
452	4.6		141		XX		70		132		8	
459	4.0		154		XX		100		67		12	
461	8.3		162		X		78		100		Yes	
467	5.2		145		XX		70		200		8	
468	5.8		142		X		33		63		8	

Number of experiments in *Groups I and II*:

	Group I: 27	Group II: 24	Total: 51
Survival, permanent:	12 (44%)	23 (96%)	35 (69%)
Survival, 1 to 36 hours:	15 (56%)	1 (4%)	16 (31%)
Survival, less than 1 hour:	0	0	0

Of the 27 dogs with unfavorable prognosis 44 per cent also survived permanently. This is in sharp contrast to the 45 control animals with unfavorable prognosis (tables 1-4) which were treated with intra-arterial transfusion alone or with ouabain and of which only 2 survived.

The long, and as yet unexplained, sequence of animals with bad prognosis mentioned previously and evident in the experiments given in table 5 starting with no. 440 (February 1948) affects the average results toward less rather than greater survival.

Despite this, it is obvious that tetraethyl ammonium chloride produced an increase in survival rates which occurred whether the outlook for survival was favorable or unfavorable. It is probable that the temporary transfusion of almost one fourth of the withdrawn blood volume, which the fall of arterial pressure after administration of tetraethyl ammonium chloride caused, had some effect upon the survival rate. However, this is probably not the sole explanation for similar transfusions given purposely to animals with unfavorable prognosis in other shock experiments at comparable periods during hypotension had no effect upon survival rate.

Comparison of the survival rates obtained in all our experiments with those of other investigators (2-5), using much the same technique to produce hemorrhagic shock, shows them to be similar in our 'early' group of experiments but markedly higher in the late control group and notably in the groups given ouabain or tetraethyl ammonium chloride. The chief difference in treatment seems to have been that other workers returned the blood by vein while we employed the artery. The advantages of retrograde intra-arterial transfusion have been reviewed elsewhere (17). In our experiments it was invaluable.

In contrast with intravenous transfusion, arterial pressure can be restored to



normal exceedingly rapidly. The volume of blood transfused is automatically determined by the pressure-volume requirements of the vascular tree. Further, less blood is needed to achieve and maintain comparable arterial pressures.

It was of interest to follow the course of the infused blood in a dog in which circulation had all but failed, especially because Kohlstaedt and Page (6, 7) had noted the fact that patients who had stopped breathing often took a deep breath within seconds of administration of blood into the femoral artery. So quick was this reaction that the blood would not have had time to complete the circuit of the body and so reached the hypoxic respiratory center. With this in mind, x-ray photographs

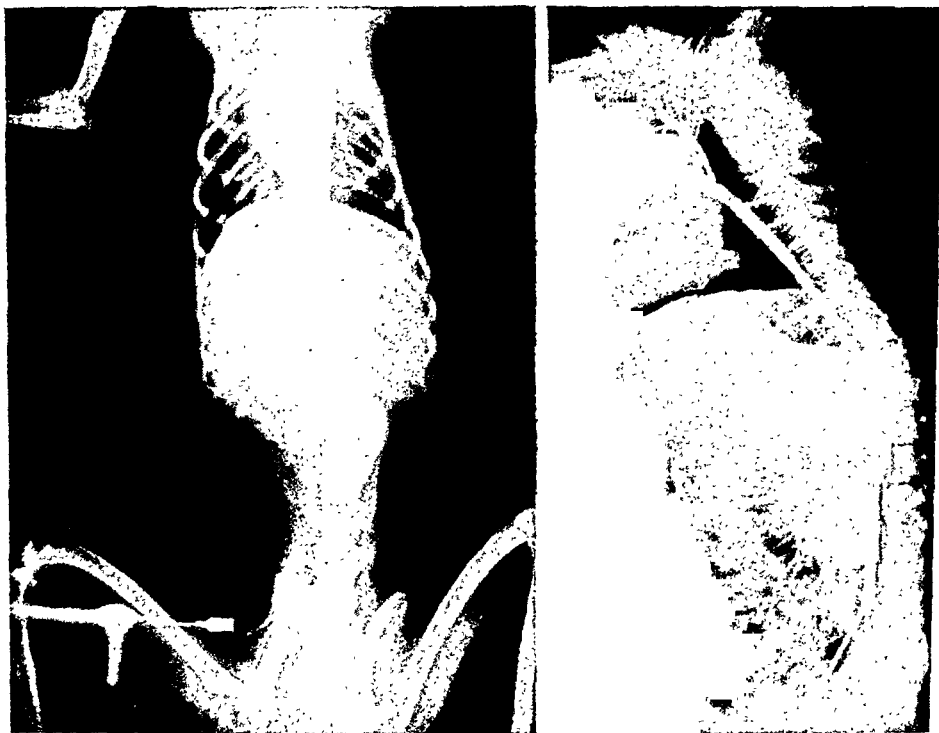


Fig. 5. INTRA-ARTERIAL INFUSION of Skioldan into the femoral artery of a dog deeply in shock with extreme hypotension. *Left.* Note that the kidneys fill first. *Right.* Same animal a few seconds later. The coronary and medullary vessels have now filled.

were taken serially as a 70 per cent solution of Skioldan was infused into the femoral artery of a dog almost dead from severe bleeding.

Figure 5 was taken a few seconds after the infusion was started and shows that the kidneys fill first, followed by filling of the coronary and spinal medullary vessels. Thus retrograde flow up the aorta occurs when the arterial pressure is low.

The rapid, controlled restoration of arterial blood pressure can be of decided importance in an emergency, many of which we encountered during the course of these experiments. With blood pressure at 30 mm. Hg, a further fall can be disastrous unless quickly countered by arterial transfusions. Intravenous administration is often too slow under such circumstances. To further prove this point in a series of experiments, we deliberately dropped the pressure to zero by withdrawing, after the shock procedure, further quantities of blood. This led to stoppage of circulation and

TABLE 6. SURVIVAL RATES AFTER HEMORRHAGIC SHOCK AND RESUSCITATION

I EXP. NO.	II BLEEDING VOL., ML/100 GM. B. WT.	III TIME OF HYPOTENSION, MIN.	IV REL. INTAKE DURING HYPOTEN.	V INTAKE DURING TRANSF., % OF BLEEDING VOL.	VI ADRENALIN RESP., % OF ORIG. RESP.	VII SURVIVAL
244	5.2	166	XX	70	50	10
245	4.9	168	X	90	0	36
246	5.4	161	0	100	30	Yes
247	5.8	138	XXX	100	100	Yes
248	6.2	179	X	100		0
250	6.2	170	XX	60	20	10
251	7.0	163	0	50	20	10
253	5.9	170	X	70	10	10
254	5.5	158	XXX	75	0	3
256	4.6	178	XX	100	100	9
259	5.0	198	X	100		0
261	7.0	167	X	100		0
262	3.4	167	X	66	65	Yes
264	5.1	152	X	75	40	10
265	7.1	154	0	40	0	10
267	5.8	225	0	87	90	Yes
268	6.3	163	0	65	75	10
271	6.0	151	XXX	82	40	3
272	6.7	176	0	60	50	Yes
274	4.0	162	X	100	0	10
275	4.4	167	0	66	5	Yes
276	7.3	190	0	54	26	Yes
277	6.9	168	0	73	44	Yes
278	3.9	175	X	100		0
281	5.2	145	0	43	40	10
282	5.7	191	X	100	50	10
283	6.9	145	0	32	33	10
291	8.4	163	0	100		0
293	5.4	167	0	100	23	10
294	6.3	168	X	100	50	Yes
318	6.0	163	XX	63	83	10
320	8.1	173	XX	57	40	10
321	8.6	157	0	58	54	Yes
325	7.0	141	X	100	100	Yes
326	4.8	136	0	53	30	Yes
333	5.8	163	X	100	100	Yes
334	4.8	166	X	100		0
335	5.5	183	X	100	56	10
337	4.5	155	X	50	75	10

No. of expts: 39. Survival, permanent: 13 (33%); survival, 1 to 36 hrs.: 20 (51%); survival, less than 1 hr.: 6 (16%).

respiration, i.e., a condition where resuscitation by means of intravenous transfusion did not promise success.

In 39 dogs already subjected to the usual hemorrhagic shock procedure, more blood was withdrawn into the pressure reservoir, until the heart and respiration stopped as indicated by electrocardiographic and pneumographic records.

Respiration usually stopped first and was started artificially in from 2 to 8 minutes. When the heart had stopped for about 2 minutes, treatment was started. This consisted of rapid intra-arterial transfusion along with 0.5 ml/kg. of 1:10,000 adrenalin, artificial respiration and ouabain (0.05 mg/kg.) intravenously. The results are presented in table 6.

Eighty-four per cent of the animals could be resuscitated by this method; 51 per cent of these lived for an average period of 10 hours and 33 per cent survived indefinitely. Thus 16 per cent failed to be resuscitated, which seems to us a small number in view of the long period of hypotension followed by stoppage of heart and respiration. An example of the records of these experiments is presented in figure 6.

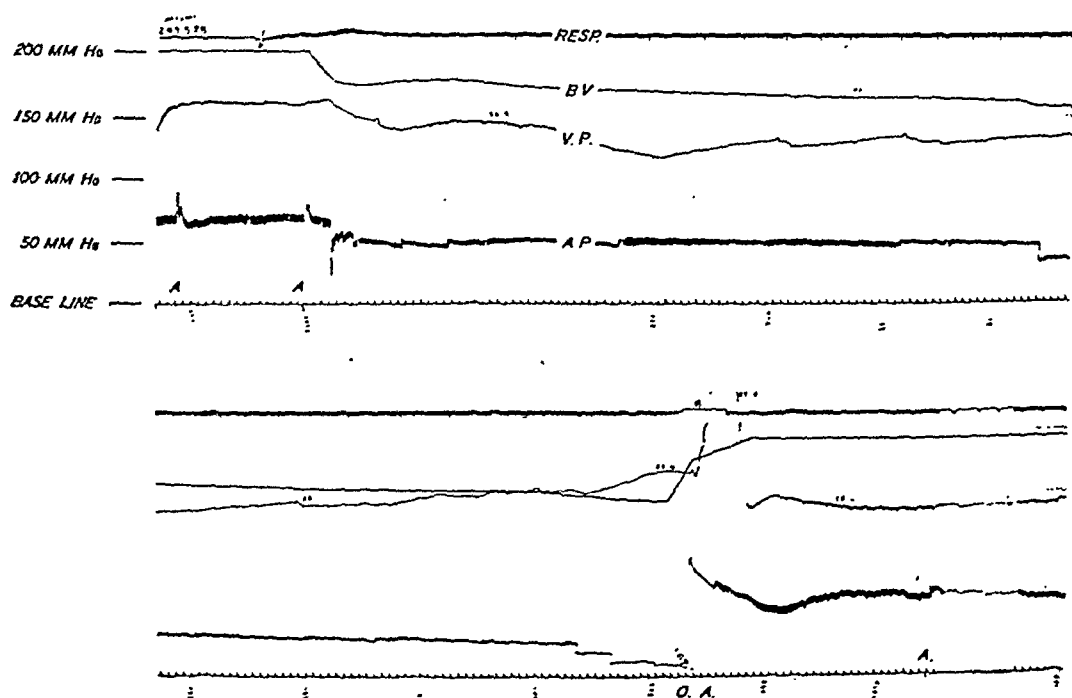


Fig. 6. RECORD OF DOG resuscitated following shock procedure and further bleeding until respiration stopped for 6 min. and the heart (as measured by the electrocardiogram) for 2½ min. Total time of the hypotension was 167 minutes. Venous pressure (V.P.) is also recorded from the right auricle.

While we have no control observation on animals, it seems reasonable to assume that arterial transfusion and artificial respiration were the chief factors in the resuscitation under these conditions of emergency. Obviously, an *intravenous* transfusion could not have been of benefit. It is important to point out that enough heparin has entered the animal's (24) circulation to reduce the possibility of coagulation and the blood returned to the animal was heparinized providing an additional safeguard against intravascular clotting.

#### SUMMARY

The effect of hemorrhage of severe grade and intra-arterial retransfusion on the survival of 244 dogs was studied. It was found possible, confirming our earlier work, to divide the animals into those with good and poor outlook for survival. The criteria selected were 1) maintenance of a steady pressure during the hypotensive period without takeup of blood from the constant pressure reservoir, 2) a relatively

small amount of the blood withdrawn being required to restore the normal level of arterial pressure and 3) no marked change in responsiveness to adrenalin after re-transfusion as compared with the control.

During the period covering about the first year of the work, the survival rate for those with poor prognosis was 7 per cent and with good prognosis 35 per cent. The next two years saw a marked increase in the number of animals with good prognosis, from 35 to 84 per cent with permanent survival with a corresponding decrease in those with bad prognosis. Survival in the latter group did not increase significantly. Periods of weeks to months occurred when for no reason we could discover, survival fell off sharply only to recover without change of technique, a phenomenon we had previously noted in scalded animals.

Administration of ouabain did not affect the survival rate in 94 animals, except possibly in the early group of experiments where over-transfusion may have adversely affected survival in the animals with good prognosis. Tetraethyl ammonium chloride significantly increased survival, 44 per cent in animals with bad prognosis and 96 per cent with good.

The value of intra-arterial transfusion in emergency is emphasized. Thirty-nine animals after being subjected to the shock procedure were bled further until breathing and heart beat stopped, the former from 2 to 8 minutes, the latter about 2 minutes. Eighty-four per cent could be resuscitated, 51 per cent lived an average of 10 hours and 33 per cent survived indefinitely.

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# MECHANISM OF THE ARTERIAL PRESSURE RESPONSE TO THE VALSALVA TEST: THE BASIS FOR ITS USE AS AN INDICATOR OF THE INTACTNESS OF THE SYMPATHETIC OUTFLOW

STANLEY J. SARNOFF, ESTHER HARDENBERGH AND  
JAMES L. WHITTENBERGER

*From the Department of Physiology, Harvard School of Public Health*

BOSTON, MASSACHUSETTS

THE details of the response of arterial pressure to short periods of high intrapulmonary pressures (40 mm. Hg) have not previously been clearly elucidated. It is the purpose of this communication to define the reflex pathways over which this response travels and to indicate its usefulness as a test for the degree of activity of the sympathetic nervous system, in the normal and after sympathectomy.

Since Valsalva first described the procedure of sustained expiratory effort against a closed glottis (1), the test has been used for various purposes.<sup>1</sup> Several years ago, it was determined in the dog that the marked rise in blood pressure following the release of a high intrapulmonary pressure did not occur if an appreciable reduction of blood volume had previously been brought about (fig. 1). At that time, the response to the Valsalva test was used as an index of the adequacy of the circulating blood volume (2). In anticipation of the study of shock patients, control data were obtained from patients with normal blood volumes on the surgical wards of the Massachusetts General Hospital. The rise in blood pressure that followed the release of a high intrapulmonary pressure was found to be present in all but the sympathectomized patients. The following experiments resulted from this observation.

Certain phases of the arterial pressure response to forced expiration have been carefully studied by Wilkins and Culbertson (3). These authors found that in man, following the Valsalva test, the overshoot of femoral arterial pressure was either diminished or abolished after bilateral sympathectomy. Direct proportionality was not established between the degree of sympathetic denervation and the diminution of the overshoot in any given patient, although the collected data suggested that such a proportionality might exist.

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Received for publication April 23, 1948.

<sup>1</sup> The various names given to this test or maneuver (clinically) are likely to give rise to confusion in regard to understanding its mechanism. It is certain that simply drawing in a deep breath and holding it does not initiate that sequence of events resulting in a uniform post-stimulus overshoot. The term 'breath-holding test' would, therefore, seem to be inadequate. Drawing in a deep breath and blowing it out as forcefully as possible is likewise not likely to yield either a striking or consistent response. Actually the proper test (as was first described by Valsalva) is a sustained, forced, expiratory effort against an obstructed airway which is usually either a closed glottis or a column of mercury. It is suggested, therefore, that the test be called either by Valsalva's name as heretofore, or the term 'forced obstructed expiration' be used.

## METHODS

Male and female dogs weighing from 9 to 15 kilograms were used. Morphine sulphate (5 to 10 mgm.) was used as preliminary medication, after which urethane was administered as needed for quiet steady anesthesia. This amounted to between 0.4 and 1.0 grams per kilogram of body weight. No anesthetic agent was administered between tests which were being compared to each other.

Arterial pressure was recorded from the femoral artery. The pressure-recording device used was an electronic strain gage led through a carrier type amplifier to a direct-writing oscillograph<sup>2</sup> as previously developed (4). The paper on which all the tracings in this experiment were made was printed in millimeter divisions. The paper speed for all experiments was 2.4 mm. per second. Intravenous, intracaval, intra-auricular and intraventricular pressures were recorded through a no. 9 catheter 100 cm. long.

In some experiments an incision was made in the atlanto-occipital membrane and a no. 6 ureteral catheter threaded down the subarachnoid space until the tip

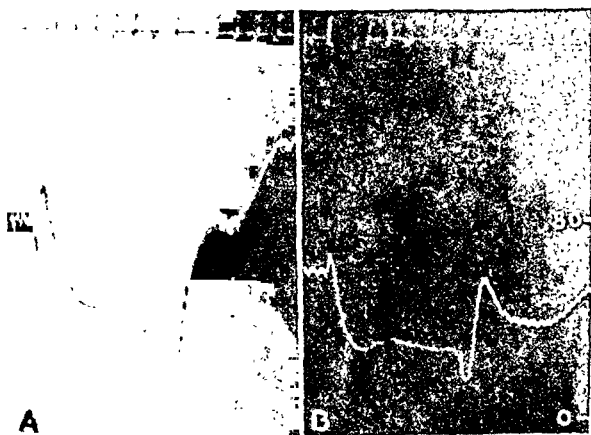


Fig. 1. ARTERIAL PRESSURE RESPONSE to intratracheal pressure of 40 mm. Hg applied for 30 seconds. Stimulus A was applied prior to bleeding. Stimulus B was applied following the removal of one sixth of the dog's estimated blood volume. Blood pressure in mm. Hg. Time marker indicates 5-second intervals. Dog is under nembutal anesthesia.

reached the sacral area. Varying doses of 1 per cent procaine hydrochloride could then be conveniently administered. As demonstrated by Co Tui (5) a total spinal anesthesia can be induced by this catheter technique. Co Tui's method was modified to produce a graded and also reversible chemical sympathectomy, since the subsequent washing out of the procaine in the subarachnoid space with saline was capable of removing the block and restoring the sympathetic influence on the periphery in a matter of minutes.

A tracheal cannula was secured in place and connected to a source of compressed air. This stream of air under pressure was run through a mercury trap so that the pressure in the system would always be at, but never exceed, 40 mm. Hg. The trap was connected to a 20-liter carboy in such a way that a large reservoir of air under a pressure of 40 mm. Hg would be available to afford an instantaneous stimulus. A side arm on the tracheal cannula allowed the dog to breathe normally between stimuli.

An ordinary sphygmomanometer cuff was wrapped snugly, but not tightly, around the dog's chest with its upper margin in the axillae. The dog was then

<sup>2</sup> Sanborn Company, Cambridge, Mass.

secured to the dog board in the supine position. A rubber tube from the cuff was attached to the source of compressed air from the carboy so that the perithoracic cuff would be inflated at the same time and by the same pressure that was applied to the lung.

The standard stimulus, used in every case, was as follows. At the onset of the stimulus, the airway was closed and the tube from the pressure reservoir opened, thus exposing the lung and perithoracic cuff simultaneously to a pressure of 40 mm. of Hg. After 30 seconds, the airway was opened and the tube leading from the pressure reservoir clamped shut, permitting immediate restoration of normal conditions of breathing. Three hundred and fifty-seven such tests were performed on 10 dogs.

When vagus section was performed, it was done high in the neck. When tetraethylammonium chloride was used, 100 mgm. in 10 cc. of saline were injected into the femoral vein in a period of about one minute.

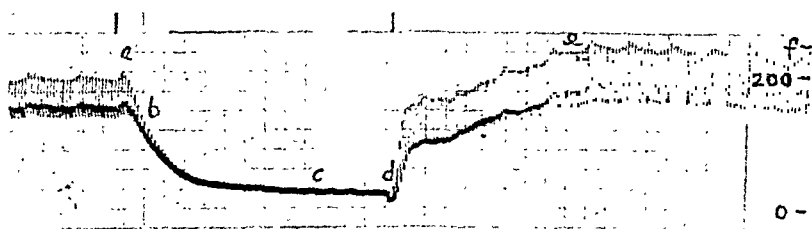


Fig. 2. TYPICAL RESPONSE of femoral arterial pressure to the Valsalva test. Components a, b, c, d, e and f explained in text. Pressure in mm. Hg at right. Signal marks indicate beginning and end of applied intratracheal pressure which lasted for 30 seconds.

## RESULTS

*Response to Standard Stimulus.* Figure 2 is representative of the arterial pressure response to the standard stimulus as described above. It can be seen that immediately following the application of the standard stimulus there is a slight rise in pressure (a), followed by a rapid fall (b) and narrowing of the pulse pressure. There is then a tendency to level off (c) with a further narrowing of the pulse pressure. Immediately after the release of intrathoracic pressure there is a slight further fall (d), and then a bounding pulse wave that climbs to levels (e) far in excess of the prestimulus figure. This component (e) will be termed the overshoot. After a variable period of time, the blood pressure returns to its prestimulus level (f).

Figure 3 shows the response of the pressure in the femoral artery, femoral vein, abdominal vena cava, right auricle and right ventricle to elevation of intrapulmonary pressure to 40 mm. Hg for 30 seconds. All tests in figure 3 were done in succession on the same dog. The vagi had previously been cut.

All but the pressure tracing from the right ventricle are self-explanatory. The irregularities in the curve during the forced expiration on all the tracings correspond in part with the dog's attempts to make respiratory motions. These same irregularities are sometimes seen in the femoral artery tracings but are less well marked.

The right ventricular pressure response to forced expiration (fig. 3E) is of some additional interest in that there was a distinct overshoot in the right ventricle follow-

ing the release of intrapulmonary pressure just as in the systemic arterial response. The significance of this will be discussed later.

*Effect of Vagus Section on the Overshoot.* Most of the experiments in this investigation were performed after the vagi had been cut. This was done for two

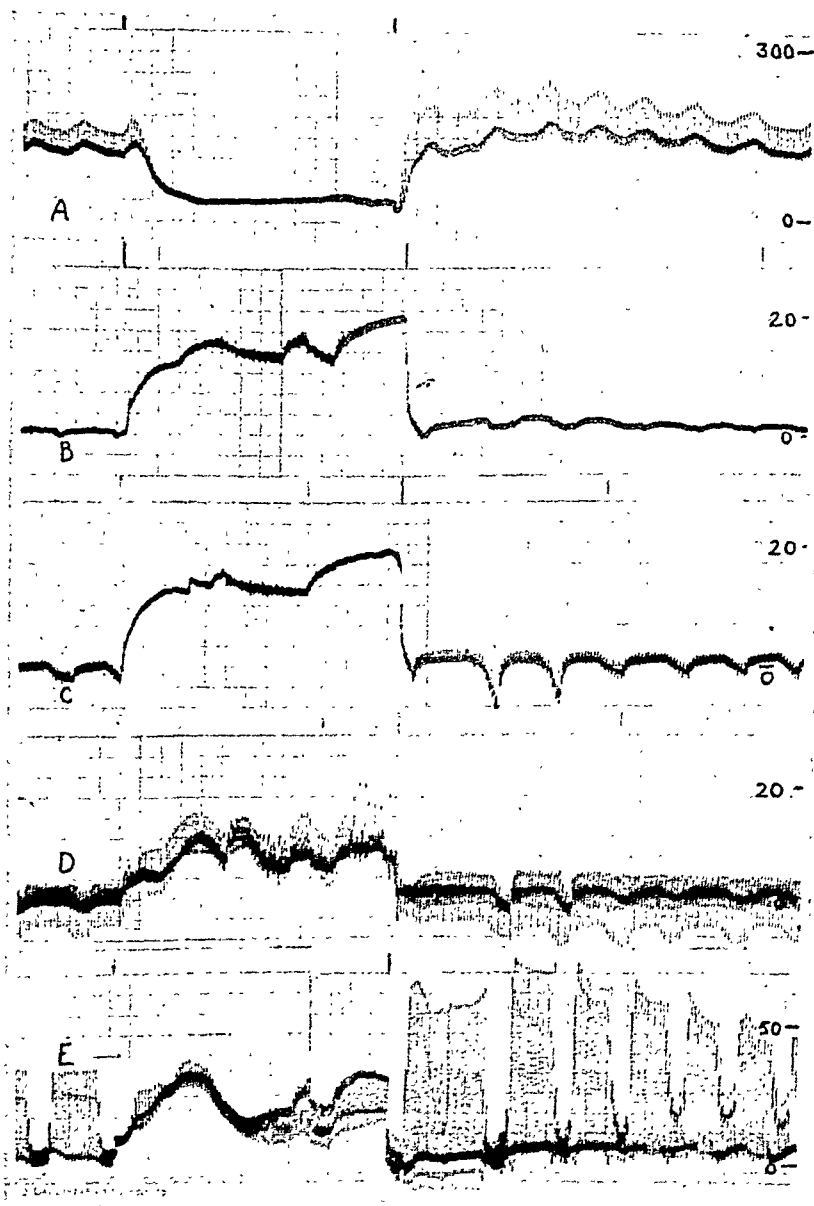


Fig. 3. RESPONSES TO THE VALSALVA TEST of the pressures in: *A*, femoral artery, *B*, femoral vein, *C*, inferior vena cava, *D*, right auricle and *E*, right ventricle. Pressure in mm. Hg designated to the right of each scale. The various ranges were obtained by changing the amplification of the current from the strain gage to suit the particular need.

reasons. First, the sectioning of the vagi yielded a steadier preparation and one free from the excessive vagal activity that usually accompanies morphine-urethane anesthesia. This made interpretation and comparison of the records easier and gave larger overshoots with which to deal, since after section of the vagi the extent of the overshoot usually increased and became more uniform. This fact led to the



conclusion that vagal activity can inhibit the extent of the overshoot. Figure 4A shows the overshoot response with intact vagi, and figure 4B, the response after the vagi had been cut. It can be seen that the overshoot response after vagus section is greater. Atropinization had the same effect. Secondly, it was thought that by cutting the vagi, the aortic depressors would be eliminated and a more satisfactory study of the influence of the carotid sinuses could be made.

*Effect of Spinal Anesthesia on the Overshoot.* Figure 5 shows the effect of gradually ascending spinal anesthesia on the overshoot (*component e*). The vagi had previously been cut. The response prior to spinal anesthesia is shown in figure 5A, where a distinct overshoot is seen. The responses obtained in the same animal, as the spinal anesthesia is driven gradually higher by additional injections of procaine, are shown in figure 5B, C and D. It can be seen that as the block ascends and the blood pressure falls, the overshoot diminishes and is finally abolished. In figure 5E is shown the effect of the rapid irrigation of the spinal canal with saline and the rise

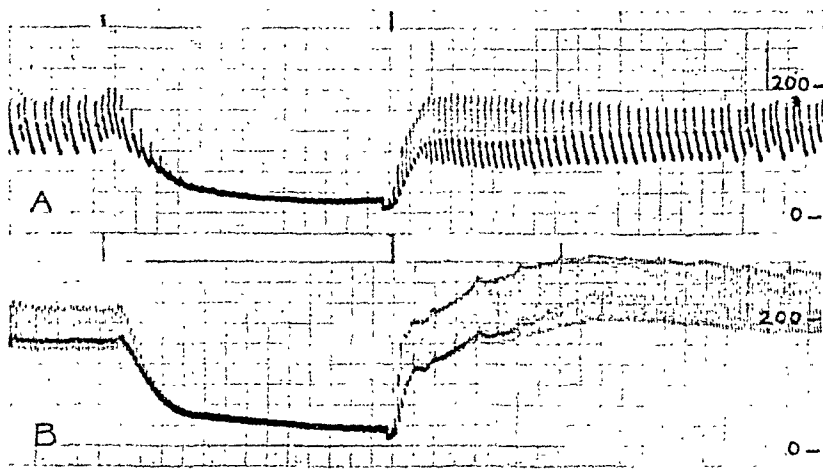


Fig. 4. EFFECT OF CUTTING THE VAGI on the arterial pressure response to the Valsalva test. A, prior to vagotomy; B, two minutes following vagotomy. Blood pressure in mm. Hg at the right.

of blood pressure to normal levels. In 5F the overshoot is again present in a test obtained after the washing. These data are representative of the results of this procedure in three dogs, in which no contrary data were obtained.

*Effect of Tetraethylammonium Chloride on the Overshoot.* Figure 6 shows the effect of tetraethylammonium chloride on the femoral arterial response to forced inflation of the lungs. Acheson *et al.* have demonstrated that this agent blocks autonomic impulses at the ganglionic synapse (6, 7). Since the vagi had previously been cut in this experiment, the effect of the drug is presumably on the sympathetic outflow. Figure 6A shows the response prior to the administration of the drug. Figure 6B shows the fall of blood pressure directly after the injection of 100 mgm. of tetraethylammonium chloride intravenously. In figure 6C it can be seen that the overshoot has been greatly diminished. In other experiments it was abolished. Figure 6C, D and E show the responses during and after recovery from the drug. These figures are representative of the results of this procedure in 5 dogs, in which no contrary data were obtained.

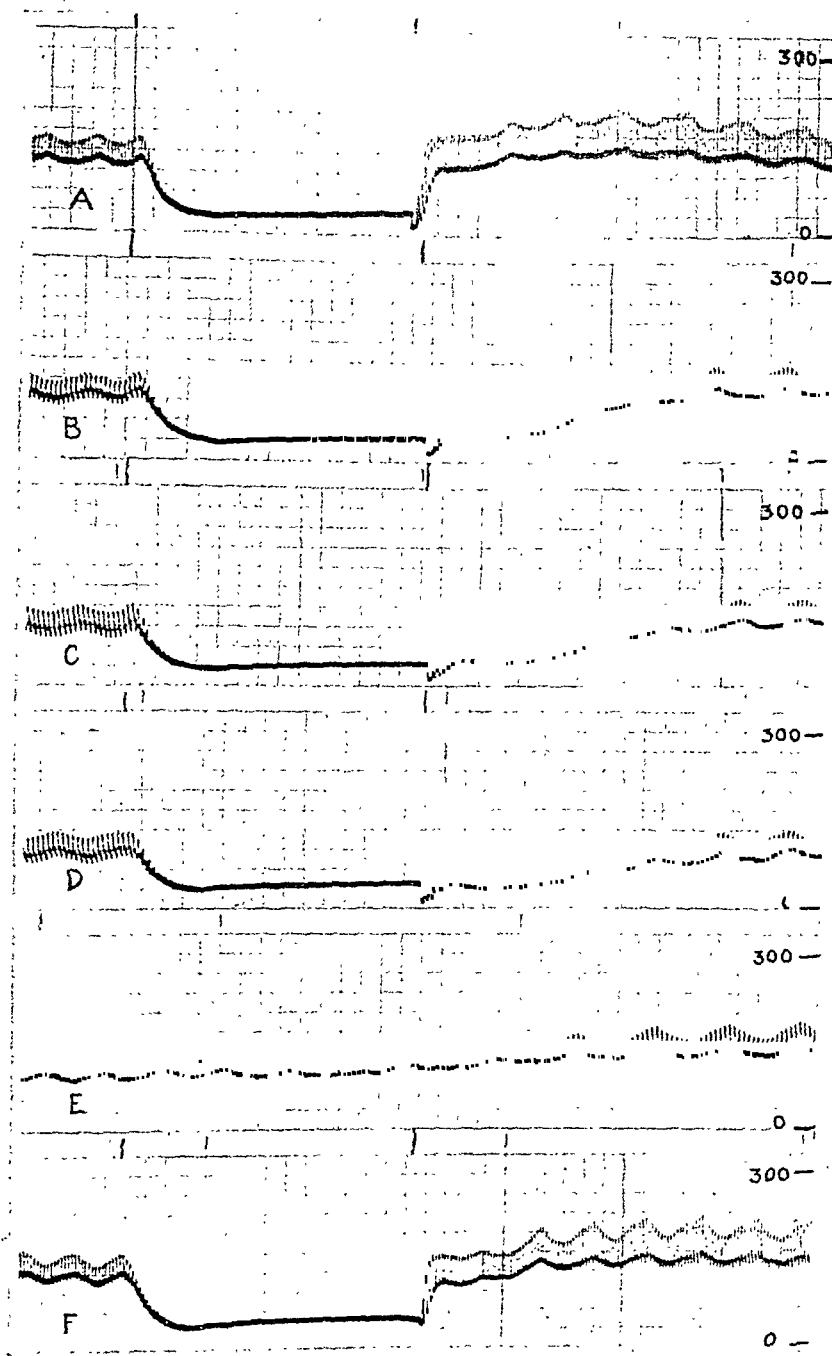


Fig. 5. EFFECT OF GRADUALLY ASCENDING SPINAL BLOCK on the arterial pressure response to the Valsalva test. *A*, normal response. *B*, response 11 minutes after injection into spinal catheter of 30 mgm. (3 cc.) of procaine; between *B* and *C* (6-minute interval) second injection of procaine, 10 mgm. (1 cc.). *C*, response 5 minutes after second procaine injection; between *C* and *D* (6-minute interval) third injection of procaine, 10 mgm. (1 cc.). *D*, response 3 minutes after third procaine injection. *E*, prompt rise in blood pressure accompanying the washing out of procaine from the spinal canal with 100 cc. of normal saline injected into catheter (signal mark in tracing *E* indicates beginning of wash). *F*, response 10 minutes after completion of spinal wash. Blood pressure in mm. Hg at right.

*Effect on the Overshoot of a Continuous Intravenous Infusion of Epinephrine.* It was thought that the effect of a continuous infusion of epinephrine might shed some

light on the efferent pathway involved in the overshoot response to the Valsalva test. For, if the overshoot were due to sympathetic activity reflexly engendered during the period of forced expiration, in the presence of excess circulating epinephrine the

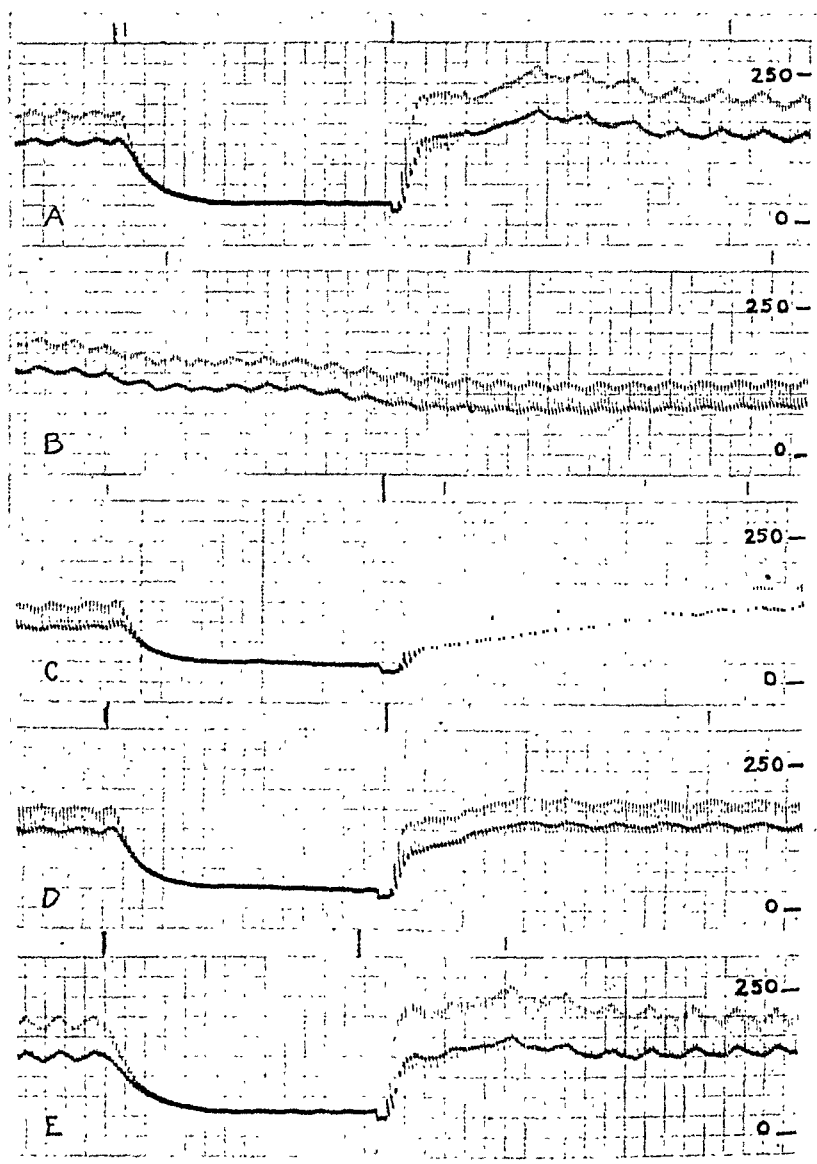


Fig 6. EFFECT OF INTRAVENOUS TETRAETHYLAMMONIUM CHLORIDE on the arterial pressure response to the Valsalva test. *A*, normal response; *B*, fall in arterial blood pressure following the intravenous injection of 100 mgm. of tetraethylammonium chloride (10 cc.) given at beginning of tracing; *C*, response 2 minutes after injection; *D*, response 6 minutes after injection; *E*, response 1 hour after injection. Blood pressure in mm. Hg at the right.

stimulus should find the efferent pathway already activated and, therefore, have little or no additional effect. Figure 7 shows the effect on the overshoot response of a continuous intravenous infusion of 0.1 cc. per kilogram per minute of a 1:10,000 solution of epinephrine hydrochloride. Figure 7A shows the response prior to the

infusion; figure 7B the response during the infusion. It can be seen that prior to the infusion the overshoot is appreciable and during the infusion it is abolished. Figure 7C shows the response after the epinephrine infusion was stopped, and it can be seen that the overshoot has returned. These figures are representative of several tests that were done before, during and after the infusion. The largest overshoot obtained during the infusion was always smaller than the smallest overshoot obtained before and after the infusion.

*Effect of Common Carotid Artery Occlusion on the Overshoot.* Figure 8 shows the effect on the overshoot of occluding both common carotid arteries. The vagi have abolition of the overshoot response during carotid occlusion. Figure 8C shows the

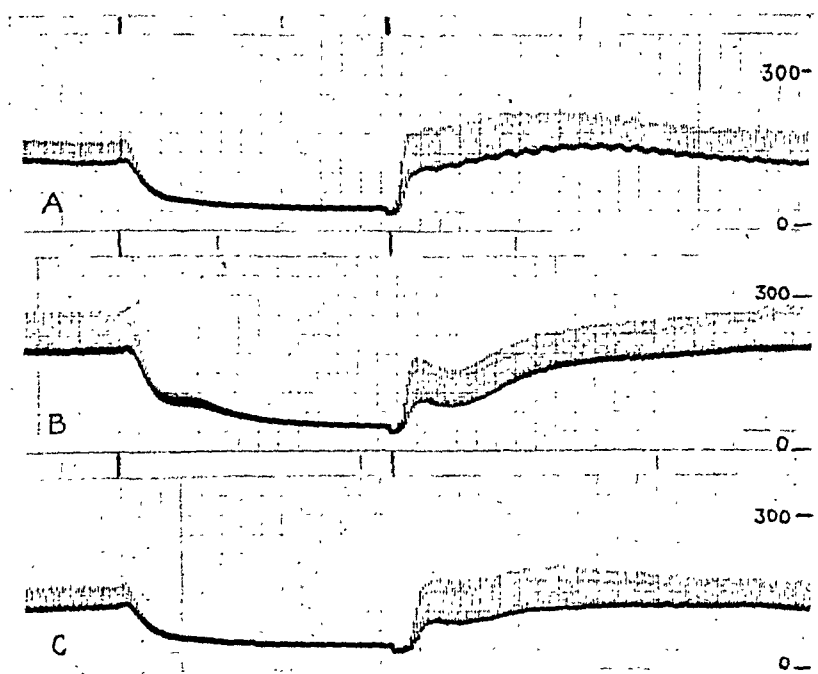


Fig. 7. EFFECT OF CONTINUOUSLY ADMINISTERED intravenous epinephrine hydrochloride on the arterial pressure response to the Valsalva test. A, response prior to infusion; B, response during a continuous infusion of 0.1 cc/kgm/min. of a 1:10,000 solution of epinephrine hydrochloride; C, response 11 minutes after stopping the infusion. Blood pressure in mm. Hg at the right.

been cut. Figure 8A shows the response with open carotids. Figure 8B shows the overshoot after release of the carotids; it can be seen that the overshoot has regained its initial extent. These data are representative of the results of this procedure in 8 dogs in which no contrary data was obtained. The overshoot was not abolished in every case but was always significantly diminished. This was observed in the intact (fig. 9) as well as the vagotomized dog. In those experiments in which the overshoot was diminished but not abolished by carotid occlusion, it was thought that a possible explanation might be as follows. The pressure in the carotid sinuses is lowered by carotid occlusion yet is high enough to be further affected (via vertebral anastomoses) by the systemic hypotension resulting from the test stimulus, thus

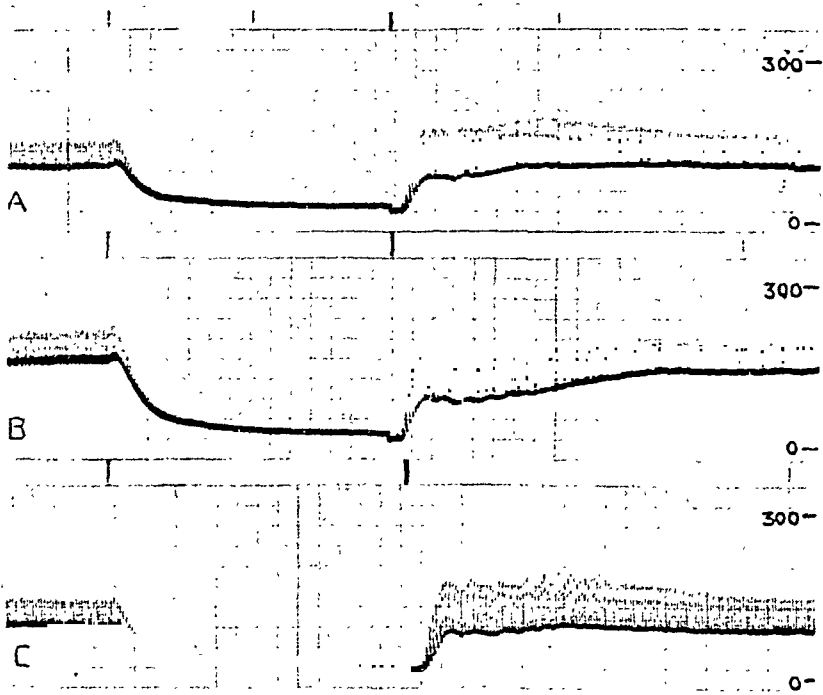


Fig. 8. EFFECT OF CAROTID ARTERY OCCLUSION on the arterial pressure response to the Valsalva test. Vagi cut. *A*, response prior to carotid occlusion; *B* response during occlusion of both common carotid arteries; *C* response after release of the carotids. Blood pressure in mm. Hg at the right.

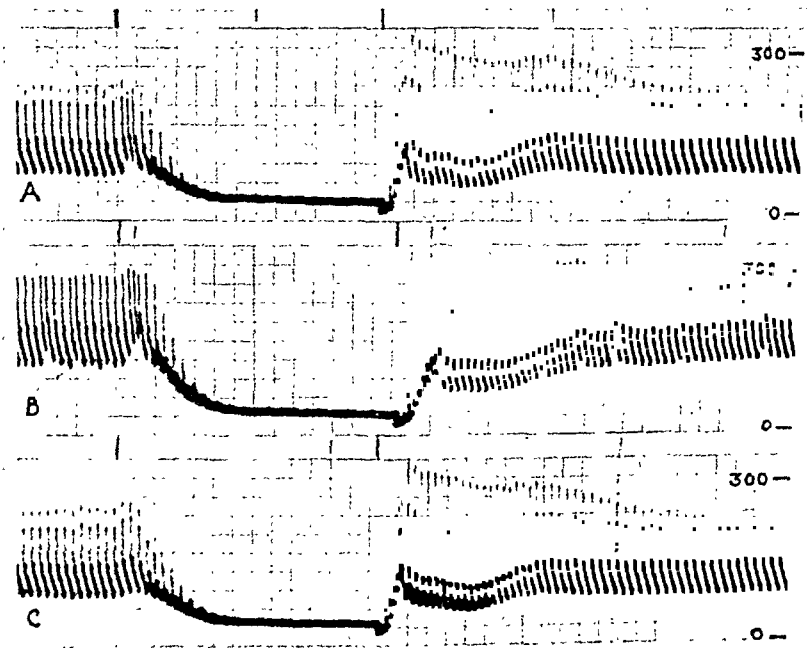


Fig. 9. EFFECT OF CAROTID ARTERY OCCLUSION on the arterial pressure response to the Valsalva test. Vagi intact. *A*, response prior to carotid occlusion; *B* response while both common carotid arteries are occluded; *C* response after release of the carotids. Blood pressure in mm. Hg at the right.

bringing into play more vasoconstrictor activity. This was verified by direct observation.

## DISCUSSION

Although factors affecting the height of the overshoot of blood pressure following the Valsalva test are the important considerations in this group of data, the explanation of the other components of the blood pressure curve during and after the application of increased intrapulmonary pressure will be briefly discussed first.

*Component a.* Immediately after the intrapulmonary pressure is increased there is a slight but definite rise in arterial pressure in some instances. At the time the stimulus is applied, the pulmonary vascular tree has a normal amount of blood in it. This sponge-like structure is then squeezed by the sudden thrust of air against the chest wall that is kept more or less fixed by external pressure. The contained blood is thus forced in the direction of least resistance, namely towards the left heart, yielding a sudden increment in left venous return, cardiac output, and, thus, an increased systemic arterial pressure. This appears to be the most logical explanation for the *a* component, even though in some dogs no *a* component was ever elicited.

*Component b.* The precipitous fall *b* is due to a diminution in venous return. An increase in intrapulmonary and intrathoracic pressure is produced and acts like a pneumatic clamp across the vessels of the pulmonary bed and the great veins in the thorax. The blockade must be almost complete and the result is a marked decline in venous return, cardiac output and systemic arterial pressure.

*Component c.* Following the precipitous fall *b*, there is a tendency for the arterial pressure to level off *c* at very low levels.

*Component d.* Immediately following the release of pressure in the airway there is a further slight fall in arterial pressure *d*. The compressed vascular tree of the sponge-like lung expands, momentarily absorbing right ventricular output, and thereby momentarily decreases venous return to the left ventricle, left ventricular output and systemic arterial pressure.

*Component e.* The bounding rise in arterial pressure to heights far in excess of prestimulus levels is the significant component in the clinical test for the degree of intactness of sympathetic vasoconstrictor pathways. The marked overshoot does not occur in the absence of the vasoconstrictor mechanism as evidenced by the experiments with spinal anesthesia and tetraethylammonium chloride. Likewise, the activity of the carotid sinuses is essential, since, if they are excluded, the response is either abolished or diminished. It seems logical to postulate, therefore, that the overshoot is a result of the following sequence of events: *a*) When the intrathoracic pressure is raised at the onset of the stimulus, the arterial pressure falls sharply and the carotid and aortic pressor receptors are exposed to greatly diminished intra-arterial pressures; *b*) the vasomotor center responds to the decreased afferent impulses and reflex pressor mechanisms are brought into action via vasoconstrictor, adrenal and cardio-accelerator fibers. If these pathways are intact, the onrushing blood released at the end of the stimulus is put out of the left ventricle more forcefully against an intensely constricted peripheral arteriolar bed and the pressure rises to extreme heights. On the other hand, if a significant portion of the peripheral vasoconstrictor mechanism is not functioning, the blood is put out against an arteriolar bed to which the vasoconstrictor impulse has not been fully applied, and the arterial

pressure does not exceed prestimulus levels if the defect in the efferent sympathetic pathway is severe enough. The extent to which the adrenal glands participate in this response has not been determined, but they might reasonably be expected to play an appreciable part. The effect of the release of accumulated metabolites on the circulation during the overshoot period has not been evaluated.

The overshoot of right ventricular pressure was at first thought to be due solely to the release of blood that had been dammed back on the venous side of the circulation during the stimulus. This interpretation would be in keeping with the experiments of Euler and Liljestrand in which carotid artery occlusion had little or no effect on the pulmonary arterial pressure of the cat under chloralose (8). It was with some surprise, therefore, that we found first a marked increase in the level of right ventricular pressure following simple bilateral common carotid artery occlusion (fig. 10). A systematic investigation of this interesting reflex response has been undertaken and will be reported separately. Secondly, the magnitude of the right ventricular overshoot was appreciably diminished if the test was performed while the carotids were occluded. This led us to believe that occlusion of the common

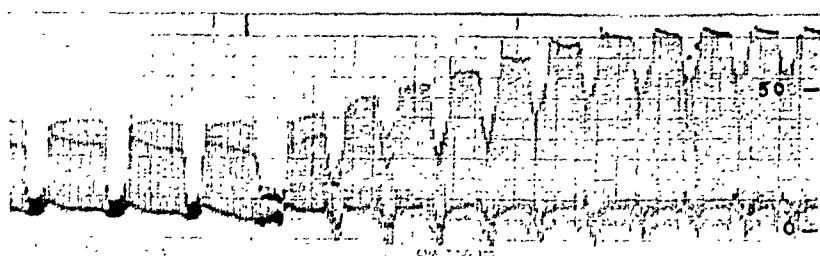


Fig. 10. EFFECT OF BILATERAL COMMON CAROTID ARTERY OCCLUSION on right ventricular pressure. The occlusion was performed at the signal. Blood pressure in mm. Hg at the right.

carotid arteries has a specific effect on right ventricular systolic pressure, elevating the resting level and also decreasing the magnitude of the overshoot response.

The effect of spinal anesthesia on the arterial pressure response to the Valsalva procedure should be evaluated in the light of recent work on the rôle of the sympathetics in the hypotension induced by spinal anesthesia. It had previously been thought that under spinal anesthesia muscular stasis might be responsible for the hypotension. However, the results of differential blocking procedures (in which the sympathetic but not the motor neurones in the subarachnoid space were blocked) indicated that paralysis of the sympathetic outflow was largely, if not completely, responsible for the fall in blood pressure under spinal anesthesia (9-12).

The extent to which extraneous factors can alter the overshoot must be considerable. It has been shown that a low blood volume can diminish or abolish it (2). Factors which cause greater than normal sympathetic activity in the prestimulus period (such as cold, fear, pheochromocytoma etc.) might be expected to alter the overshoot by pre-exciting the efferent sympathetic pathways before the test is performed. Either excessive vagal activity or medication interfering with vagal action might alter the overshoot by either augmenting or diminishing the normal

inhibitory action of the vagus. Likewise, hypersensitivity of the carotid sinuses logically falls in that group of causes that might be expected to alter the response to the Valsalva test.

#### SUMMARY

1. Pressure changes in various parts of the circulatory system, resulting from a 30-second elevation of intrapulmonic pressure, form characteristic and consistent patterns.

2. The arterial pressure response has six components, of which the poststimulus overshoot, or rise of blood pressure above the prestimulus level, is the main subject of this paper.

3. This overshoot is the result of reflex sympathetic activity engendered by the hypotension present in the carotid sinuses during the period that venous return is impaired. (The extent of participation by the aortic receptors was undetermined.)

4. Removal or diminution of carotid sinus activity diminishes or abolishes the overshoot.

5. Partial or complete blockade of reflex sympathetic activity either by tetraethylammonium chloride or by graded, segmental, spinal anesthesia diminishes the overshoot *in direct proportion to the extent of the blockade*.

6. Vagal activity inhibits the overshoot to a variable degree.

7. The presence of an excess of circulating epinephrine diminishes and may abolish the overshoot.

8. The overshoot of right ventricular systolic pressure is influenced by the presence or absence of carotid sinus activity, much as is the overshoot of systemic arterial pressure.

9. The standardization of any clinical test designed to use the degree of overshoot as a quantitative estimate of sympathetic activity should take into consideration the factors of medication, vagal activity, cold, apprehension, carotid sinus sensitivity and the presence of circulating epinephrine, as well as the patient's blood volume.

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# CARDIAC EFFECTS OF INTRAVENOUS INJECTION OF SMALL VOLUMES OF STRONGLY HYPERTONIC SOLUTIONS<sup>1</sup>

WILLIAM W. WALCOTT AND INGRITH JOHNSON DEYRUP

*From the Department of Physiology, College of Physicians and Surgeons and the Department of Zoology, Barnard College, Columbia University*

NEW YORK CITY

THE intravenous injection of even small volumes of strongly hypertonic solutions may result in marked circulatory changes in mammals. Numerous studies have been carried out in an attempt to analyze these effects, particularly with reference to the therapeutic uses of such injections. Among the vascular and cardiac changes which have been described in cats, dogs and human beings are generalized vasodilatation (1-3), coronary arterial constriction (4), alterations in the electrocardiogram, and tachycardia (3, 5). We have noted in a series of experiments on cats and dogs that the effects of such injections upon the heart may be more complex than described previously. The cardiac alterations include irregularities, dropped beats or extrasystoles, and, perhaps most characteristically, a precipitous and profound slowing of the heart, which is abolished by bilateral section of the vagus nerves. The latter effect may be demonstrated with ease in cats and, less readily, in dogs as well. The purpose of this paper is to describe in some detail the cardiac effects of the intravenous injection of small volumes of strongly hypertonic solutions.

## METHODS

Experiments were performed on 20 cats ranging in weight from 1.7 to 4.0 kg. and 13 dogs varying from 3.5 to 15 kg. in weight. Most of the animals were anesthetized by the intravenous or intraperitoneal injection of nembutal (sodium pentobarbital—36 mg/kg.). In addition, experiments were carried out on 3 dogs which had been decerebrated under ether anesthesia, upon one unanesthetized (local 2% procaine anesthesia), and on 2 chronically sympathectomized dogs.

In general, simultaneous femoral arterial pressure tracings were made using a standard recording mercury manometer and a Hamilton metallic membrane manometer with optical recording. Heart rates and information as to the general character of the pulse waves were obtained from the Hamilton manometer records. The error in calculating the heart rate from counts of the number of pressure pulses seen on the Hamilton record, during successive three-second intervals, was estimated as being of the order of  $\pm 5$  per cent. The mercury records were used to relate the mean arterial pressure with the observed cardiac rate changes. Electrocardiogram records were obtained by the use of a General Electric portable electrocardiograph.

The effects of the injection of 20 per cent sodium chloride solution were studied in detail. In a few additional experiments, records were also taken during the intravenous administration of other hypertonic solutions including 5, 10 and 15 per cent NaCl and 50 per cent glucose. The volumes of these fluids given in a single injection ranged from one to 6 ml. for cats, and from 2 to 16 ml. in the observations upon dogs. All of the solutions were at room temperature. Equal volumes of 0.9 per cent NaCl were given in several experiments as controls on the mechanical and thermal effects of this type of fluid infusion. The results of injecting comparable volumes of distilled water and of approxi-

Received for publication July 9, 1948.

<sup>1</sup> Supported in part by the Baruch Fund for Physical Medicine.

mately isotonic solutions of low and of high pH (0.155 M. hydrochloric acid and 0.155 M. sodium hydroxide) were studied also.

Injections were made into the external jugular vein, or less frequently, into the femoral vein or one of the auricular appendages which had been exposed by opening the chest during intermittent positive pressure ventilation of the lungs. Solutions were administered as rapidly as possible through 20-gauge needles inserted into rubber-capped cannulae tied into the blood vessels. The rates of injection varied from one to 4 ml. per second and were timed accurately by closing a key in a circuit so that a light flashed on the Hamilton record and a signal magnet writing on the mercury record was activated. In the later experiments, the key was fixed upon the end of the plunger of the injection syringe and thus was pressed automatically at the start of the injection. Using the injection signal obtained in this manner on the manometer records, the latency of the response to any given solution was measured as the time from the beginning of the injection to the beginning of the first cardiac cycle of a series showing prolongation.

### RESULTS

*Observations on Cats.* In 7 of 12 experiments on cats, transient cardiac irregularities were observed within a second after the start of the injection of 2 to 3 ml. of 20 per cent NaCl. The irregularities were seen on the Hamilton records as

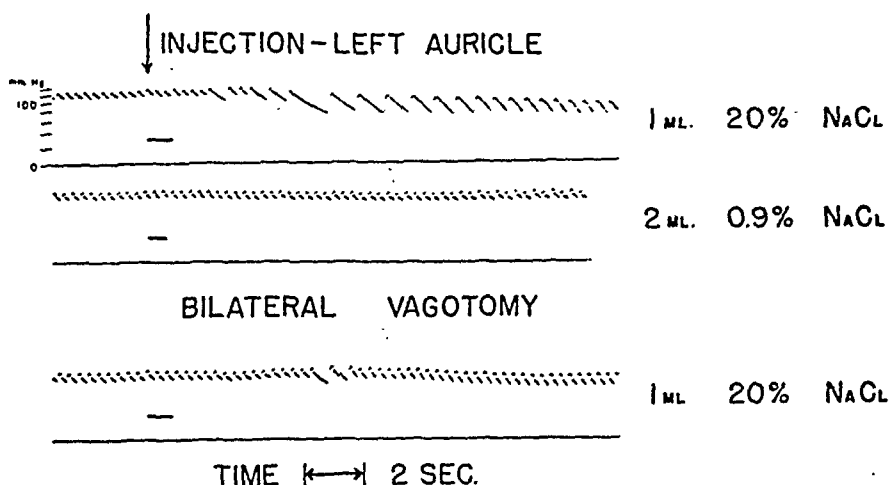


Fig. 1. Cat 12. NEMBUTAL ANESTHESIA. Femoral arterial pressure records showing the characteristic bradycardia following the rapid injection of 20% NaCl and its absence after bilateral vagotomy. All injections into cannulated left auricular appendage.

shortened cardiac cycles with an occasional cycle of unusually long duration. Immediately after this brief period of irregularity, or occurring even in its absence, was a far more consistent and striking cardiac change. This was marked slowing of the heart, as illustrated in figure 1 from a typical experiment. Such bradycardia was noted in all experiments upon nembutalized cats, as shown by analysis of both Hamilton manometer and electrocardiographic records. It commenced within 1.8 to 4.0 seconds (av.  $2.34 \pm 0.78$  sec.) following intrajugular injection and in three experiments resulted in complete cardiac arrest for three to nine seconds. In other instances the minimum heart rates observed, which occurred within 3 to 21 seconds after injection into the external jugular vein, varied between 22 and 63 per cent of the control rate. A transient and moderate to marked lengthening of the PR interval (by 20 to 100% of the duration of the control PR interval) was noted at this time in the three experiments in which ECG records were obtained. The bradycardia lasted for an average of 107 seconds, the duration ranging in all experiments

from 36 seconds to 6 minutes. At the end of this period, the heart rate returned to or surpassed the pre-injection level. Thus in four experiments transient tachycardia, in which the heart rate rose to a peak 12 to 20 per cent higher than the control rate, followed the phase of bradycardia. It may be noted at this point that the mean arterial pressure fell more or less parallel with the decline in heart rate. The arterial pressure did not return to the control level as rapidly as did the heart rate, and the slight tachycardia seen several minutes after the injection of hypertonic NaCl may have resulted from reflex adjustments to the concomitant hypotension.

Following bilateral cervical vagotomy, the pattern of heart rate changes in response to 20 per cent NaCl injection was altered markedly (fig. 1). Usually some cardiac irregularities occurred after the injection, but true bradycardia was not observed in any of the experiments after the vagi had been sectioned. In three experiments a slight and brief decrease in heart rate was noted, yet this cardiac slowing was of a totally different order of magnitude from the effect with the vagi intact. The duration was not more than three to nine seconds, and the slowing was of relatively lesser degree, the lowest rate observed under these conditions being 80 per cent of the control rate. Marked alterations in the PR interval were not noted after bilateral vagotomy. On the other hand, slight but significant tachycardia occurred in five experiments following the injection of 20 per cent NaCl in vagotomized animals. In two cases the heart rate increased to 26 and 31 per cent of the control, and the elevation in rate lasted for 30 and 45 seconds, respectively. In spite of the fact that marked bradycardia did not occur, the mean arterial pressure decreased in vagotomized cats. Usually the hypotension was of moderate degree, however, the fall in pressure averaging 42 mm. Hg less than the fall occurring when the vagi were intact. In only one experiment was the fall in mean arterial pressure found to be greater after than before vagotomy, when identical volumes of 20 per cent NaCl were injected into identical regions of the vascular bed.

The control injections of isotonic (0.9%) NaCl produced negligible changes in heart rate in both vagotomized and intact animals. In seven experiments, the heart rate was observed to vary within  $\pm 11$  per cent of the control level during the first 15 to 18 seconds after the injection, a period during which striking bradycardia always occurred after injection of the strongly hypertonic solutions. In four experiments a transient and questionable rise or fall in mean arterial pressure ( $\pm 5$  to 6 mm. Hg) was noted, but it lasted only during and for one to two seconds after the injection.

Table 1 summarizes the results of six experiments in which hypertonic solutions other than 20 per cent NaCl were injected. These solutions included 15 and 10 per cent NaCl and 50 per cent glucose (approximately isotonic with 10% NaCl). That the bradycardia observed was a result of the hypertonicity of the injected fluid, rather than a specific effect of excess concentration of sodium (or chloride) ion upon the heart may be concluded from the fact that the injection of 50 per cent glucose caused marked bradycardia just as did hypertonic NaCl. The response to the former solution was somewhat less striking, but it was quite comparable quantitatively with the cardiac slowing following the injection of 10 per cent NaCl. Thus, in some animals marked decrease in heart rate followed the injection of 2 ml. of 20

per cent NaCl, whereas no bradycardia was seen after the injection of 2 ml. of 10 per cent NaCl or 50 per cent glucose. In these animals, however, the heart rate slowed after injection of twice this volume of 10 per cent NaCl or 50 per cent glucose. It may be noted from table 1 that 15 per cent NaCl regularly produced bradycardia which was qualitatively similar to, though less marked than the slowing following injection of 20 per cent NaCl. In preliminary tests it was found that injection of 5

TABLE 1. COMPARISON OF THE EFFECTS OF THE INTRAVENOUS INJECTION OF VARIOUS HYPERTONIC SOLUTIONS IN ANESTHETIZED CATS

EXPER. NO.	SOLUTION INJECTED	VOL.	SITE OF INJECTION	RATE OF INJECTION	VAGAL BRADYCARDIA		
					Latency	Max. degree <sup>1</sup>	Duration
		ml.		ml/sec.	sec.		sec.
4	20% NaCl	1	L. J. V.	1.5	—	Absent	—
	"	2	"	1.5	2.3	46	28
	"	2	"	0.4	4.0	62	19
	50% Glucose	4	"	1.3	4.5	50	24
29	20% NaCl	2	Rt. Aur.	1.2	1.8	13	34
	15% NaCl	2	"	3.3	0.9	5	16
	10% NaCl	2	"	2.0	—	Absent	—
	"	4	"	4.2	1.2	3	22
	50% Glucose	2	"	2.5	—	Absent	—
	"	4	"	4.7	0.8	3	21
55	20% NaCl	2	L. J. V.	2.9	2.0	2.6	19
	15% NaCl	2	"	2.7	1.6	1.8	13
	10% NaCl	2	"	2.4	—	Absent	—
	50% Glucose	4	"	2.9	2.7	1.9	20
56	20% NaCl	2	L. J. V.	1.8	2.1	2.1	26
	15% NaCl	2	"	1.9	3.2	1.2	12
	10% NaCl	2	"	2.4	—	Absent	—
	50% Glucose	2	"	1.7	—	Absent	—
	"	6	"	2.1	1.4	1.4	25
57	20% NaCl	3	L. J. V.	2.1	2.4	2.8	26
	15% NaCl	3	"	2.3	—	Absent	—
	10% NaCl	3	"	2.3	—	Absent	—
	50% Glucose	6	"	2.6	—	Absent	—
58	20% NaCl	3	L. J. V.	2.1	3.0	35	48
	15% NaCl	3	"	2.3	3.0	20	55
	10% NaCl	3	"	2.5	4.4	1.2	13
	50% Glucose	6	"	1.3	—	Absent	—

<sup>1</sup> Maximal degree of vagal bradycardia = duration of most prolonged cardiac cycle following injection ÷ duration of average control cardiac cycle.

per cent NaCl did not result in bradycardia. Specific tests of the effects of varying the rates of injection of the hypertonic solutions were not made. It may be noted, however, that volumes of 10 and 20 per cent NaCl which caused marked bradycardia when administered rapidly could be introduced very slowly and caused no alteration in heart rate. In general, it would appear that the concentration, volume and rate of injection of hypertonic solutions are interrelated variables determining the occurrence and intensity of the bradycardia response.

Of the other fluids tested, distilled water, a 'maximally hypotonic solution', did not alter the heart rate in the quantities used (2 to 4 ml.). Similarly one ml. volumes of the strongly alkaline solution, approximately isotonic sodium hydroxide, were without effect. The intravenous administration of one ml. of isotonic hydrochloric acid caused a characteristic and totally different type of response in the five experiments in which it was tested—delayed hypotension, apnea, and occasionally a slight bradycardia. It may be concluded then that the injection of highly hypertonic solutions produces a profound slowing of the heart rate in anesthetized cats with vagi intact and that this effect is quite specific and dependent upon the increased concentration of the injected fluid. It has been observed also that such injections may result in cardiac irregularities even after bilateral vagotomy. In addition, there is transient hypotension which is exaggerated by the concomitant vagal bradycardia, but occurs even in the absence of any change in heart rate. The overall response is, however, more complicated than this. Alterations in the depth and rate of respiration occur and will be described in more detail in a subsequent paper. The vari-

TABLE 2. VAGAL BRADYCARDIA IN UNANESTHETIZED AND DECEREBRATED DOGS RESULTING FROM THE INTRAVENOUS INJECTION OF 20 PER CENT SODIUM CHLORIDE

EXPER.	VOL.	RATE	VAGAL BRADYCARDIA		
			Latency	Max. Degree <sup>1</sup>	Duration
	ml.	ml/sec.	sec.		sec.
989 Unanest.	8	2.7	3.8	1.5	7
	12	2.6	5.0	12	30+
D1 Decer.	8	2.9	3.0	8	7
D2 Decer.	4	1.8	4.5	38	82
D3 Decer.	3	2.1	5.3	3	8

<sup>1</sup> Maximal degree of vagal bradycardia = duration of most prolonged cardiac cycle following injection ÷ duration of average control cardiac cycle.

ations in cardiac rate, mean arterial pressure and respiration appear to be independent to a large extent and, yet without doubt, each is modified by the others through reflex cardiovascular and respiratory adjustments.

*Observations on Dogs.* The results obtained in the seven experiments which were carried out on normal dogs anesthetized with nembutal were in marked contrast to the effects seen in nembutalized cats. Instead of the consistent and profound bradycardia dependent on the presence of intact vagi, as described in the latter, the alterations in heart rate were inconstant. In three experiments the heart rate was slowed by less than 11 per cent and a comparable degree of bradycardia occurred after bilateral vagotomy. Similarly, some decrease in heart rate was noted after vagotomy in two of the four experiments in which irregularities with more marked slowing of the heart had occurred when the vagi were intact. In these four experiments, the minimum heart rates observed ranged between 37 and 77 per cent of the control rates. In most of the experiments there was a profound fall in arterial pressure and, closely correlated with this, a tachycardia which occurred within 30

to 36 seconds in six of the seven experiments upon normal nembutalized dogs. Such tachycardia was absent in one of the two experiments carried out on chronically sympathectomized dogs anesthetized with nembutal. In the other sympathectomized animal, however, tachycardia was observed even after bilateral vagotomy and may, therefore, be tentatively ascribed to direct action of the hypertonic agent on the heart.

As they stand, these results indicate that the vagal bradycardia following the injection of hypertonic solutions, as seen in cats, does not occur in the dog. It has been shown, however, that anesthesia with barbiturates reduces the cardio-inhibitory action of the vagi in mammals (6, 7), and consequently four additional experiments were carried out upon dogs under conditions such that anesthetic effects were eliminated as far as possible. The experiments were conducted on one unanesthetized dog (local procaine anesthesia) and three preparations in which decerebration had been performed under ether anesthesia, and the ether subsequently removed. In all of these experiments, marked bradycardia was elicited by the rapid injection of 3 to 12 ml. of 20 per cent NaCl. In latency, duration and intensity, this bradycardia was quite comparable to the cardiac slowing seen in cats following administration of hypertonic solutions (table 2). The bradycardia was, moreover, abolished by bilateral cervical vagotomy. The injection of corresponding volumes of 0.9 per cent NaCl was found to be without measurable effect on the heart rate or arterial pressure. Thus the specific response to the injection of hypertonic solutions was closely similar to the effects previously observed in cats. As in the latter species, rather complex respiratory and arterial pressure alterations following the injection of hypertonic NaCl were noted as well.

#### DISCUSSION

Transient arterial hypotension following the injection of small volumes of strongly hypertonic solutions has been described by a number of investigators (1, 4, 8-11), but these workers have not noted the occurrence of marked vagal bradycardia in cats and dogs after such injections. Sollman (12) cited Retzlaff as stating that the injection of larger volumes of hypertonic solutions caused a fall in blood pressure and speeding of the heart rate followed, in later stages by cardiac slowing as a result of stimulation of the vagus center. On the other hand, Kisch (2) noted that in anesthetized cats the slowing of the heart, which resulted from injection of one cc. per kg. body weight of hypertonic salt and sugar solutions, was not mediated by the vagi since the pattern of circulatory changes was unaffected by vagotomy. Bernstein (1) and Binet and Stoicesco (8) did not describe bradycardia in etherized and chloralosed dogs and Bernstein stressed rather the occurrence of tachycardia which he attributed to both vagal and sympathetic mechanisms. Gennari and Levi (10) found that one cc. of 20 per cent NaCl per kg. body weight injected into anesthetized dogs caused hypotension lasting for a period of 30 seconds or more and that this phenomenon was not affected by atropine or vagotomy. Their published records give no evidence of bradycardia. Similarly, Muirhead *et al.* (4) observed that the intravenous injection of 10 per cent NaCl, 50 per cent glucose and

25 per cent albumin in dogs anesthetized with sodium pentobarbital was without significant effect on the heart rate, although transient arterial hypotension occurred. The fact that such a constant and striking phenomenon as the vagal bradycardia described in the present paper has not been noted previously may, perhaps, be referred to the predominant use of anesthetized dogs as subjects for studies on the cardiovascular effects of intravenous administration of hypertonic solutions. Evidence from the experiments which we have performed on unanesthetized and decerebrated dogs as compared with nembutalized animals indicates that the occurrence of vagal bradycardia is abolished readily by anesthesia in the dog. It is far more easily demonstrated in the cat. At present, no evidence exists as to whether a similar phenomenon occurs in other species. It may be noted that it has not been described in anesthetized rabbits (13), nor in studies on unanesthetized human patients (3, 5). In the latter, ECG alterations and tachycardia may be observed to follow rapid intravenous injections of hypertonic solutions.

The significance of the vagal bradycardia described above is two-fold. On the one hand, a wide variety of hypertonic solutions—sodium chloride, glucose and other sugars, radio-opaque materials, etc.—are injected intravenously in certain clinical procedures and techniques of experimental physiology. Such infusions, if made at a sufficiently rapid rate, may have brief but profound cardiovascular effects which should be taken into account in carrying out the procedures and in interpreting the results of experiments in which they have been used. In addition the existence of this stable and characteristic response immediately raises the question of its physiological origin. Whether direct stimulation of the medullary vagus center occurs or whether the effect is attributable to reflex vagal excitation, it is highly specific in nature. Thus the response is dependent on increased concentration of the administered fluid, whereas alterations of tonicity in the opposite direction (distilled water) or of  $pH$  (isotonic acid and base) are without comparable results. Simple mechanical and thermal effects appear also to be excluded as factors participating in the origin of the response. An attempt has been made to establish the mechanism of the phenomenon and the results of this study will be presented in a subsequent paper.

#### SUMMARY

The intravenous injection of small volumes of strongly hypertonic solutions (10 to 20% NaCl, 50% glucose) results in marked alterations in the heart rate of cats and dogs. Cardiac irregularities occur initially and in nembutalized cats and unanesthetized or decerebrated dogs these irregularities are followed by transient but profound bradycardia which is absent after bilateral cervical vagotomy. This pattern of response is highly modified in dogs under nembutal anesthesia for, in these circumstances, relatively less or variable alterations in heart rate occur and true vagal bradycardia is absent. The species variation in the effects of nembutal upon this circulatory response mediated by the vagi obscures the fundamental similarity of the phenomenon in cats and dogs, for in both species profound vagal bradycardia can be demonstrated reproducibly under specifically defined conditions.

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# MECHANISM OF VAGAL CARDIAC SLOWING FOLLOWING INTRAVENOUS INJECTION OF SMALL VOLUMES OF STRONGLY HYPERTONIC SOLUTIONS<sup>1</sup>

INGRITH JOHNSON DEYRUP<sup>2</sup> AND WILLIAM W. WALCOTT

*From the Department of Zoology, Barnard College, and the Department of Physiology, College of Physicians and Surgeons, Columbia University*

NEW YORK CITY

**A**MONG the circulatory effects of the intravenous injection of small volumes of strongly hypertonic solutions, one of the most striking is a transient but profound bradycardia. Thus, within a few seconds after the rapid injection of 2 to 8 ml. of 20 per cent NaCl, 50 per cent glucose, or other highly concentrated solution into the veins of cats and dogs, the heart rate slows. This effect, which may last for several minutes, is abolished by bilateral section of the vagus nerves. It is present in nembutalized cats and in unanesthetized or decerebrate dogs, but not readily demonstrable in dogs anesthetized with nembutal (1). The experiments described in the present paper were carried out in order to analyze the mechanism of this vagal bradycardia as seen in anesthetized cats following the injection of strongly hypertonic solutions.

## METHODS

Experiments were performed on 37 cats ranging in weight from 1.5 to 4.0 kg. Usually the animals were anesthetized with nembutal (36 mg/kg. intraperitoneally), but in a few experiments decerebration was performed under ether anesthesia and the ether was then removed. The trachea was cannulated and, in some experiments where intrathoracic structures were exposed, artificial respiration was administered by means of a small adjustable pressure pump. Simultaneous Hamilton manometer and mercury manometer pressure tracings were obtained from the two femoral arteries.

In general, heart rates were determined with an estimated error of  $\pm 5$  per cent from the Hamilton manometer recordings by counting the number of cardiac cycles occurring during three-second intervals. In eight experiments, respiration was recorded by means of a chest pneumograph.

Injections of hypertonic solutions were made as rapidly as possible (1-4 ml/sec.) and timed accurately as described in a previous paper (1). The solutions used included hypertonic NaCl (15 and 20%) and glucose (50%). Control injections of isotonic (0.9%) NaCl were made as well. In a few experiments, sodium cyanide (0.4 to 2 mg. per kg. dissolved in 2 to 3 ml. of 0.9% or rarely 20% NaCl) was injected into the external jugular vein and the latency of the resulting gasp reflex was measured with a stop watch. The sites of injection of the hypertonic fluids included the external jugular veins, femoral veins, ascending aorta, and auricular appendages.

Experiments were carried out to determine whether the vagal bradycardia resulted from direct stimulation of the medullary vagus center, or from excitation of peripherally located receptors resulting in reflex vagal discharge. In the first series of experiments, a comparison was made of the occurrence and latencies of the responses following injection into peripheral veins, the heart, and the ascending aorta. Furthermore, the latent periods for the vagal bradycardia, following injection of 20 per cent NaCl and the gasp reflex resulting from NaCN injection into the same vein, were compared. In a second series of experiments, tests were made for the occurrence of vagal bradycardia after excluding the possibility of direct action of the hypertonic agent on the medullary

Received for publication July 9, 1948.

<sup>1</sup> Supported in part by a grant from the Baruch Foundation for Physical Medicine.

centers by isolation of the head from the arterial supply of the rest of the animal. Two techniques were used. In three experiments, pairs of animals of approximately equal weight were anesthetized and their chests opened while artificial respiration was maintained. The innominate and left subclavian arteries were doubly cannulated and connected by plastic tubing so that blood flowed from the ascending aorta of one cat into the innominate and left subclavian arteries of the other cat. Similarly, the innominate and left subclavian arteries of the first animal were perfused with blood from the ascending aorta of the second. In this way, the arterial supply of the head of one cat was obtained from the body of the other and the separate effects of injection of the hypertonic agent upon the body and upon the perfused head of each animal were tested. A second method for differentiating the peripheral as compared with the medullary effects of the hypertonic solutions was to expose and temporarily occlude the innominate and left subclavian arteries just prior to the intravenous injection of the hypertonic solution.

To determine whether the vagal bradycardia was dependent on afferent nerve fibers in the cardiac sympathetic nerves, four experiments were carried out in which injections were made before and after acute bilateral removal of the sympathetic chains from the stellate ganglia to T<sub>7</sub>. In an attempt to analyze the rôle of the two vagi in the bradycardia, the left vagus only was sectioned in four experiments and injections were then made into the left atria. Subsequently the right vagus was sectioned and the results of hypertonic salt injection following this procedure were compared with the effects after unilateral vagotomy. Atropine sulfate (1-2 mg/kg.) was injected intramuscularly and the effect upon the vagal bradycardia was observed in six experiments. Finally, injections were made into 4 animals decerebrated under ether anesthesia, in order to determine whether any central nervous structures anterior to the medullary vagus center were necessary for the occurrence of bradycardia in response to the injection of hypertonic solutions.

It may be noted at this point that the vagal cardiac slowing can be abolished by non-specific factors, such as hypotension following opening of the chest, and the excessive manipulation of the lungs which may occur during removal of the thoracic sympathetic chains. When the technique for any given procedure was being developed, negative results were obtained occasionally, but these were not given the same weight as the positive findings obtained after the experimental techniques had been perfected. In the few experiments in which cardiac irregularities were observed, a response was considered to be true vagal bradycardia only if it involved the prolongation of many successive cardiac cycles and if it was abolished or greatly modified by bilateral vagotomy.

## RESULTS

In the 16 experiments in which hypertonic solutions were injected into various regions of the circulation, it was found that bradycardia occurred consistently after injections into the femoral and jugular veins and both right and left atria, but it was either not observed at all, or was seen only after a long latent period when injected into the ascending aorta. This is illustrated in figure 1, which reproduces the Hamilton manometer records from a typical experiment. It may be noted in this figure that the heart rate decreased following all injections of 20 per cent NaCl except for the injections into the ascending aorta. The control injections of 0.9 per cent NaCl were without effect on the heart rate. After bilateral vagotomy the hypertonic injections produced slight cardiac irregularities, but true bradycardia was absent. Table 1 presents a summary of the latencies of the vagal responses seen after injections into different regions of the circulation. In addition, the latent periods of the gasp reflex following the introduction of sodium cyanide into the external jugular vein are given in the table. The consistent occurrence of cardiac slowing after intravenous and intracardiac injections is notable, as is also the exceedingly brief latency (usually less than 2 sec.) of the responses following injections into the right and left atria. The latency of the bradycardia after left jugular injection (av.

2.3 sec.) was slightly longer and the response to femoral venous injection had a yet longer average latent period (4.9 sec.). In the single experiment in which bradycardia was observed after intra-aortic injection, the latency was 3.9 seconds, or approximately twice the average latency of response to injections into the right and left atria. The brevity of the latencies of the bradycardia resulting from jugular

CAT 18 ♀ 1.7 Kg.

NEMBUTAL — 40 MG./KG.

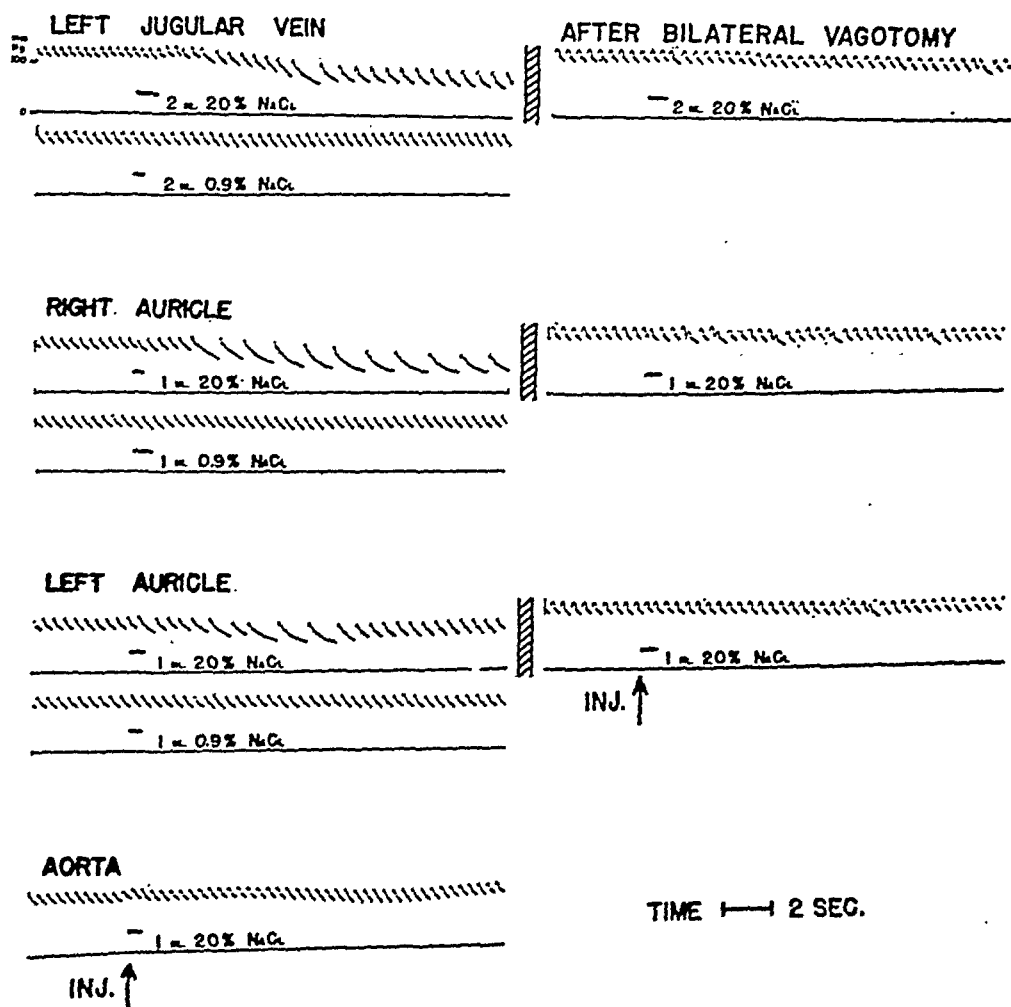


Fig. 1. Cat 18. NEMBUTAL ANESTHESIA. Femoral arterial pressure records showing the occurrence of bradycardia following the injection of 20% NaCl into the left jugular vein, the right and left auricles but not following injection into the ascending aorta. This response is abolished by bilateral vagotomy.

and cardiac injections suggests strongly that the structures stimulated by the hypertonic solutions are not as far from the site of injection as the medullary vagus center. This is further confirmed by comparison of the latency of the hypertonic salt bradycardia and the gasp reflex following cyanide injection. It may be seen from table 1 that the average latent period for the cyanide gasp reflex is  $6.2 \pm 2.1$

seconds. This latency probably represents, for the most part, the time required for the cyanide-containing fluid to pass from the jugular vein, through the right heart, the pulmonary vascular bed, left heart, and up to the carotid bodies (2). If the bradycardia which follows the injection of hypertonic solutions results from direct stimulation of the medullary vagus center, it would be expected that its latency would be longer than the latency of the cyanide gasp reflex. That such is not the case is suggestive evidence that the effect of hypertonic solutions is not exerted directly upon the medullary vagus center.

As further and more direct evidence for this hypothesis, vagal bradycardia was elicited in two out of three experiments following intravenous injection of 20 per cent NaCl into animals in which the entire arterial supply to the head was obtained by cross-circulation from another animal, and in four out of six experiments when injections were made intravenously immediately after occluding the arterial supply to the head (innominate and left subclavian arteries). It may be noted that in this series of experiments there was a fairly high proportion of negative experiments in which, after isolation of the head from the rest of the circulation, typical vagal bradycardia could not be demonstrated. This may, perhaps, be attributed to poor

TABLE 1. LATENT PERIODS OF VAGAL BRADYCARDIA AFTER INJECTION OF 1 TO 3 ML. OF 20% NaCl INTO DIFFERENT REGIONS OF THE CIRCULATION AND OF THE GASP REFLEX FOLLOWING INTRAVENOUS INJECTION OF NaCN

	LATENCY OF BRADYCARDIA AFTER INJECTION OF NaCl INTO				LATENCY OF GASP REFLEX AFTER INJECT. OF NaCN INTO LEFT JUGULAR VEIN
	Femoral Vein sec.	Jugular Vein sec.	Right Atrium sec.	Left Atrium sec.	sec.
Av.	4.9(3) <sup>1</sup>	2.3(18)	1.8(8)	1.6(8)	6.2(11)
Standard Deviation		0.9	0.3	0.7	2.1

<sup>1</sup> Numbers in parentheses indicate number of observations from which averages were obtained.

physiological condition of the medulla in these experiments, for the procedures used were, without question, somewhat radical. In any case, the positive experiments, in which vagal bradycardia was observed after exclusion of the head from the circulation of the body, show that the characteristic bradycardia may occur quite independently of direct medullary excitation by the injected solution.

Since the hypertonic solution vagal bradycardia could not be attributed to direct stimulation of the medullary vagus center, it was concluded that it was reflex in character, resulting from excitation of peripheral receptors. Injection into the left atrium usually resulted in marked bradycardia, but intra-aortic injection did not. This suggests that the receptors concerned are located, at least in part, within the heart itself and, more specifically, in the left heart. As the latent period of the response was approximately the same regardless of whether the injections were made into the right or left sides of the heart, it seems improbable that the response from injection on the right side was completely dependent on receptors in the left heart, for this should lengthen the right side latent period by the duration of the pulmonary circulation time. It is probable, therefore, that similar receptors are located in both the right and the left sides of the heart.

Figure 2, which represents data obtained from one of four similar experiments, illustrates the fact that bilateral removal of the sympathetic chains from the stellate ganglia to T<sub>7</sub> did not abolish the vagal bradycardia in response to hypertonic salt injection. Such partial sympathectomy should interrupt all cardiac afferent fibers running with the sympathetic nerves and, consequently, it was inferred that the afferent fibers from the receptors stimulated by the hypertonic solutions are not present in the sympathetic nerves and must be carried by the vagi. In four experiments, section of the left vagus nerve alone did not abolish, although it did reduce, the bradycardia resulting from the injection of hypertonic NaCl into the left atrium. This indicates that the vagal afferent fibers from the cardiac sensory endings must be bilateral in their distribution.

The intramuscular injection of atropine may completely abolish the vagal bradycardia. This result was obtained in two experiments on decerebrated cats

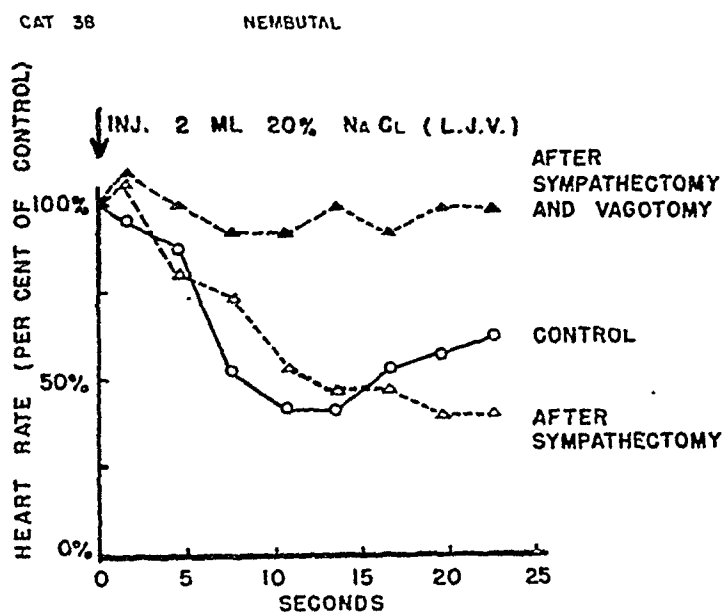


Fig. 2. Cat 38. NEMBUTAL ANESTHESIA. Showing the occurrence of bradycardia before and after bilateral removal of the thoracic sympathetic chains from the stellate ganglia to T<sub>7</sub>. Following bilateral cervical vagotomy, injection of 20% NaCl did not cause slowing of the heart rate.

injected with one mg. atropine sulfate per kg. and in cats under nembutal anesthesia injected with 2 mg. per kg. In additional experiments on 2 nembutalized animals which received one mg. per kg., the vagal bradycardia was not completely abolished, although it was markedly reduced. It was concluded from these experiments that there is no evidence for sympathetic participation in the observed bradycardia and that the efferent, as well as the afferent, path of the reflex arc is carried by the vagus nerves. Since it was found in four experiments that the vagal bradycardia could be elicited readily in the decerebrate preparation, the medullary vagus center must be adequate for completion of the reflex arc. It was concluded, therefore, that this reflex has the following neural components: *a*) receptors located, at least in part, within the left heart and probably on the right side of the heart as well; *b*) afferent fibers in the vagus nerves; *c*) the medullary vagus center; *d*) efferent fibers in the vagus nerves.

It may be noted that marked alterations in respiration occur concomitantly with the cardiac rate changes described. In general, these respiratory effects differed in character depending on whether the vagus nerves were intact or sectioned. In the animals with intact vagi, respiration was invariably arrested in expiration within a time interval ranging in eight experiments from 2.8 to 4.8 seconds (av. time, 3.4 sec.) after the injection. Usually the expiratory arrest preceded the characteristic fall in mean arterial pressure, whereas it succeeded by a fraction of a second the beginning of bradycardia. It lasted a variable length of time (3 to 43 sec.) and was followed, in general, by respiration which was irregular and frequently of increased depth. After vagotomy, expiratory arrest occurred in only one out of nine experiments, but respiration tended to become irregular and deepened, with prolongation and often exaggeration of the inspiratory phase. In general, these respiratory changes did not begin for seven seconds or longer and they were almost invariably preceded by the characteristic fall in blood pressure. Although the mechanism of the respiratory variations following hypertonic sodium chloride injection has not been analyzed in detail, the brief latency of the response and its occurrence preceding mean arterial pressure changes suggest strongly that it, too, is reflex in character. That such reflex modifications in respiration are dependent on afferent fibers running in the vagus nerves is suggested by the fact that the response is highly modified and frequently abolished by bilateral vagotomy. The slower and irregular changes noted after vagotomy may result from direct stimulation of the respiratory centers or from reflex stimulation arising from pressoreceptors of the carotid sinuses.

#### DISCUSSION

Evidence has accumulated that various chemical stimuli may excite intracardiac receptors with resultant vagal discharge. Jarisch and his co-workers (3-6) showed that the profound bradycardia and hypotension following the injection of several drugs (veratrine, mistletoe extract, histamine etc.), ions (potassium, rubidium etc.) and other substances may be attributed to the stimulation of intracardiac vagal receptors. It has been suggested by Jarisch and his collaborators that all of the agents found to cause this response (von Bezold effect) may produce cellular damage and release of ionic potassium. This potassium may be the fundamental stimulus for the observed bradycardia. A related phenomenon, perhaps, is the marked vagal slowing of the heart which results from the intravenous injection of homologous or heterologous serum in cats, as described by Brodie (7). The latter response was ascribed by Brodie to the stimulation of the pulmonary receptors of vagal afferents, but it resembles closely, in many respects, the responses described by Jarisch and the effect which we have observed. Our experiments do not answer directly the question as to whether the receptors, which mediate the response to hypertonic salt injection, are identical with previously described intracardiac sensory endings. It may be noted that the reflex effects resulting from veratrine injection, as described by Jarisch, included profound hypotension which was not entirely dependent on the bradycardia, since it occurred even after reflex cardiac rate changes had been abolished by atropinization. Such was not the case in our experiments, in which there was no consistent difference between the arterial blood pressure curves of

atropinized animals preceding and following bilateral vagotomy. It seems highly improbable that the receptors stimulated by hypertonic solutions are identical with the intracardiac stretch receptors described by Bainbridge and others (8, 9). Effective stimulation of these stretch receptors, located in the great veins and in the atria of both sides of the heart, results in reflex speeding of the heart rate. Exactly the opposite effect occurs following adequate stimulation of the receptors under consideration in the present experiments. Furthermore it may be re-emphasized that negative results following control injections of isotonic NaCl exclude the possibility of any mechanical component in this reflex.

The physiological rôle of the reflex patterns related to intracardiac receptors is not clear at present. It appears highly probable, however, that the heart, pulmonary bed and great veins may function in addition to the carotid-aortic chemoreceptor and pressoreceptor zones as regions of intravascular sensitivity to chemical and mechanical stimuli. The studies of Nettleship (10), Nonidez (11, 12) and others have provided histological evidence for a rich intracardiac receptor system in the mammal. Further, Amann and Schaefer (13) have demonstrated that centripetally travelling action potential bursts may be recorded from some of the cardiac branches of the vagi. Thus there is evidence both for the anatomical existence and for the physiological activity of intracardiac receptors. If their role is still far from being understood, it may not mean that they are of little physiological significance, but rather that the vascular adjustments which they mediate are delicate and occur in antagonism to, or in cooperation with, other reflex vascular changes.

#### SUMMARY

An analysis has been made of the mechanism of the profound vagal bradycardia following the intravenous injection of strongly hypertonic sodium chloride solutions in nembutalized and decerebrated cats. This vagal bradycardia does not result from direct stimulation of the medullary vagus center, but rather is of reflex origin. The receptors excited are located, at least in part, within the heart itself and the vagus nerves constitute both the afferent and efferent paths of the reflex arc.

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# NERVOUS CONTROL OF THE CERVICAL PORTION OF THE ESOPHAGUS

KAO HWANG, M. I. GROSSMAN, AND A. C. IVY

*From the Department of Clinical Science, University of Illinois College of Medicine*

CHICAGO, ILLINOIS

IN A recent communication (1) it was pointed out that in a dog after bilateral section of the vagus nerves just posterior to the level of the larynx the peristaltic activity of the upper third of the esophagus was preserved, as evidenced by balloon examination as well as roentgenological findings (fig. 1a, b). Consistent observations have been made by one of us (K. H.) in two more dogs. The current conception that the cervical portion of the esophagus is supplied by the recurrent laryngeal branches of the vagus would thus seem doubtful. The present study was therefore undertaken to investigate the specific motor supply to this portion of the esophagus in various animals.

Most modern textbooks of physiology and some monographs (2-7) state or imply that the recurrent laryngeal branch of the vagus is the motor nerve to the cervical portion of the esophagus. In some others either there is no definite statement (8) or it is simply said that the esophagus is supplied by esophageal branches of the vagus (9-11). All the statements in this regard are made without reference to the species of animal. Most of the old textbooks give no description of the nerve supply to the esophagus. But in the *Textbook of Physiology*, edited by Schafer (12), Starling stated that the upper part of the esophagus in man, rabbit and guinea pig is innervated by the recurrent laryngeal nerves, while in the horse and dog it is supplied mostly by a small branch of the pharyngeal nerve. However, he gave no reference to support the latter statement and no similar descriptions at all appear in the textbook written by himself (13).

The more recent workers are rather unanimous in the opinion that the cervical portion of the esophagus is supplied by the recurrent laryngeal branch of the vagus nerve and no mention is made about the earlier reports which held a view different from the current one. We were not aware of these discrepancies between the earlier reports and the more recent ones until the present experimental work had been almost concluded and the early literature was traced.

In 1836 Cooper (14) noticed distension of the esophagus with food material in rabbits after ligation of the vagus nerves in the neck. But the first extensive study of the relation of the vagus nerve to the esophagus was probably made by Reid (15) in 1838. He studied several species of animals and in the dog he noticed contraction of the pharyngeal muscles as well as the upper part of the esophagus on irritation of the pharyngeal branch of the vagus.

Chauveau (16a) made an extensive study in the horse, donkey, dog, lamb, cow and rabbit. He found that while the lower portion of the esophagus is supplied by the terminal branches of the vagus in all these animals, the upper portion is by the recurrent branches in the rabbit but in all the other animals by a long branch from the pharyngeal nerve, which, he states, was not known before. Brief descriptions of the innervation of the esophagus in different species also appear in his book (16b). He found (16c) that section of the vagi in the neck causes paralysis of the entire esophagus of the rabbit but only the lower portion in the dog and horse, while section of the 'superior motor nerve' of the esophagus in the horse involves the entire tracheal portion of the tube, which, however, does not affect deglutition seriously. Espezel (17) also noticed the difference of the innervation of the esophagus of the rabbit and the dog. He states that he is the first one to give a detailed description of the course of the 'inferior pharyngeal nerve', which is the motor supply for the upper portion of



the esophagus of the dog. He concluded that this nerve originates from the superior cervical sympathetic ganglion.

The same nerve was found in the dog by Kahn (18), but he prefers to call it the esophageal nerve. However, in regard to the origin of this nerve, he believed that it arises from the superior pharyngeal branch and also the nodose ganglion of the vagus nerve and received fibers from the superior cervical ganglion. He found in the cat, in accord with Reighard and Jennings (19), a pharyngo-esophageal nerve arising directly from the vagus trunk above the nodose ganglion or together with the superior laryngeal nerve. In the monkey (*Macacus* and *Cynocephalus*) he reported an esophageal branch from the superior laryngeal nerve. In all these animals he found, however, that the recurrent branch of the vagus gives motor innervation to a varying portion of the cervical part of the esophagus.

The results obtained by these workers in regard to the double innervation of the cervical portion of the esophagus have never been accorded the attention they deserve by the later investigators in this field. Kahn's work was quoted by Cannon (20, 21) and Inaoka (22) but received no confirmation nor comment regarding innervation of the cervical portion of the esophagus other than the recurrent. On the other hand, Inaoka's conclusion that the recurrent laryngeal nerve is the motor nerve to the cervical esophagus of the dog was supported by later workers (23-25). More recent work in the cat (26, 27) also is not in accord with Kahn's report.

Anatomists have noticed that the inferior pharyngeal branch of the vagus sends filaments to the upper part of the esophagus in the dog (28) and the cat (19), although another book (29) fails to describe them. In the monkey (*Macaca mulatta*) the cervical portion is believed to receive branches from neither the pharyngeal nerves nor the superior laryngeal nerve but from the recurrent (30). Recent work by Coulouma and Varseveld (31), based on dissection of 12 species of mammals, also made a general conclusion that the cervical portion of the esophagus is supplied by the recurrent or its branches.

Stoppage of barium meal in the upper part of the esophagus following extirpation of the superior cervical ganglion in the dog has been reported (32, 33). Knight (27) also observed in the cat that stimulation of the stellate ganglion increases the contraction of the upper third of the esophagus caused by vagal stimulation. However, Inaoka (22) concluded that the sympathetic nerves have no importance in the esophagus of mammals. Recent study in dogs after bilateral thoracic or complete sympathetic ganglionectomy has revealed no change in the activity of the esophagus (1).

#### EXPERIMENTAL

The early part of the present investigation was devoted to confirming in acute experiments that the recurrent laryngeal nerve is not essential to the activity of the cervical portion of the esophagus in the dog and to ascertain the real motor innervation.

Two preliminary experiments were made in dogs under nembutal anesthesia. The cervical portion of the esophagus was freely exposed by midline incision, resection of the sternohyoid and sternothyroid muscles and removal of a portion of the trachea, care being taken not to injure the recurrent laryngeal nerves. Faradic stimulation of the peripheral end of the vagus of either side resulted in strong tetanic contraction of the whole thoracic portion of the esophagus and the lowermost inch of the cervical portion. This is well evidenced by the fact that a finger put in the lower part of the esophagus through the cardia experienced during the vagal stimulation a strong grip by the esophageal musculature and simultaneously the cardia was passively pulled upward. This upward movement is in accord with the report by Rall, Gilbert and Trump (34); apparently the longitudinal and the circular fibers contract simultaneously. The cervical portion of the esophagus remained flaccid, showing no contraction of its musculature except its lowermost inch, and was mechanically pulled downward toward the thoracic cavity. Direct stimulation of the recurrent laryngeal

nerve at the base of the neck had no apparent effect on the esophagus although movement of the larynx was observed.

The findings above provided encouragement to proceed further to find the specific motor innervation of this portion of the esophagus. In the first experiment faradic stimulation of the superior laryngeal nerve on either side resulted in a weak but definite contraction of the whole cervical portion of the esophagus. In the second experiment no effect at all on the esophagus was observed by a similar procedure. Explanation for this discrepancy was found later. However, in this second dog faradic stimulation of a small nerve crossing the superior laryngeal nerve dorsally and then lying on the dorso-lateral aspect of the pharynx caused a very strong tetanic contraction of the whole cervical portion of the esophagus as well as the inferior pharyngeal constrictor muscle. The contraction was so strong that the cervical portion of the esophagus was, on stimulation of this nerve, immediately converted into a firm muscular cord comparable to the effect on the thoracic portion of the esophagus of stimulating the peripheral end of the vagus nerve. In the later experiments described below this nerve was consistently found and always manifested the same physiological action, and is believed to be the main, if not the only, motor innervation of the cervical portion of the esophagus in the dog. The anatomical course and the physiological significance of this nerve will be given below.

In all the experiments except the first two just described above ether anesthesia through tracheotomy was used. An audio oscillator (Hewlett Packard) was used for stimulation, the stimulating current used being adjustable in intensity and 10 to 50 cycles per second in frequency. This audio oscillator has the advantage of being adjustable to give a minimal effective stimulation, thus avoiding the effect due to the spread of the current. In some experiments the chest was opened along the midline of the sternum and artificial respiration was employed. The esophagus was then carefully exposed from the beginning at the level of the cricoid cartilage to the level of the diaphragm by excision of the lungs of the left side and division between ligatures of the left common carotid and subclavian arteries and then the aorta at the places where they cross the esophagus. Apparently there was no impairment of the excitability of the lower thoracic portion for at least one hour after the aortic transection, as judged by observations before and after this step of the operation. As a matter of fact, the slight depression of the animal as shown by the sluggishness of the corneal reflex after excision of the lungs on the left side, probably due to the resulting anoxemia, generally disappeared after the aortic arch was ligated. The beneficial effect obtained by this procedure is probably due to the improvement of the cerebral circulation. Similar experiments were done on the rabbit, cat and monkey.

In some dogs and cats chronic experiments were carried out by bilateral sectioning of this small nerve with sterile technique. Then roentgenological examinations by the aid of meals containing barium sulfate or balloon examinations as described in a previous communication were used (1).

#### 1. *Anatomical Description of the Motor Supply to the Cervical Portion of the Esophagus*

Acute experiments have been done in dogs, cats, rabbits, monkeys, guinea pigs and rats. The course of the special motor nerve supply to the cervical portion of the

esophagus was mostly made out by careful dissection in the anesthetized animal with the help of electric stimulation. It should be emphasized here that, in general, the distal portion of the nerve overlying the esophagus is so closely attached to it and often breaks up into such fine branches that the actual length of the esophagus supplied by this nerve cannot be predicted precisely by means of gross dissection but only by electric stimulation. Furthermore, the anatomic course of this nerve may undergo some variations which also can only be ascertained by the stimulation method.

a) *Dog*. In 48 experiments there was found consistently on each side a nerve which supplies the inferior constrictor of the pharynx and the entire cervical portion of the esophagus. This nerve is about one third of the size of the superior laryngeal nerve and is found dorsal and almost at a right angle to the latter at a level just above the upper border of the thyroid cartilage. It goes posteriorly and medially to lie on the dorsolateral aspect of the lower part of the pharynx. Then it courses posteriorly, in a slightly zigzag way, toward the lateral aspect of the esophagus, where it breaks up into several fine branches. Along its course after crossing the superior laryngeal nerve it was found to give a communicating branch to the external branch of the latter in about one half of the experiments. Stimulation of the external branch of the superior laryngeal after it receives this communicating branch to the external branch gives rise to a weak or moderate tetanic contraction of the cervical portion of the esophagus. This explains the result obtained in one of the preliminary experiments described before. Occasionally this nerve breaks up just above or a little below the superior laryngeal nerve into two branches, medial and lateral, which reunite before it goes to the esophagus. Fine branches are given to the inferior constrictor muscle of the pharynx from the nerve trunk or from its medial branch when it is present. When it is traced cephalad, it goes laterally and slightly dorsally, crossing the ventral surface or the lower border of the superior cervical sympathetic ganglion, where in the great majority of cases strong fibrous tissues bind it closely to the ganglion and also to a small nerve given off by the ganglion to the carotid sinus. Then it soon joins the superior pharyngeal nerve to form a short common trunk which enters into the vagus nerve just above the nodose ganglion. It is hard to make sure by gross dissection whether the sympathetic ganglion gives fibers to this nerve or not, but stimulation of the ganglion gave no apparent change of the esophagus. Occasionally this nerve has no connection with the branch of the sympathetic ganglion at all, or it may break up into two branches, of which only one comes into relation with that of the sympathetic. They unite as usual before they go to the esophagus.

As a general rule this nerve can be easily identified as a small nerve lying on the dorsolateral aspect of the lower part of the pharynx slightly dorsal to the external branch of the superior laryngeal nerve. It is present on both sides in all experiments except one, in which this nerve was not found on the left side but was present normally on the right.

According to its physiological action so far ascertained it seems appropriate to designate this nerve as the pharyngo-esophageal nerve.

The effect of the recurrent laryngeal nerve on the cervical portion of the esophagus as determined by stimulation of the peripheral end of the vagus or the recurrent laryngeal nerve at the base of the neck is rather variable. In the majority of the

experiments there was contraction of the lower fourth or third of the cervical portion of the esophagus while the upper part was not affected. Stimulation of the pharyngo-esophageal nerve in these cases usually gave rise to contraction of the whole cervical portion, which is about one third of the entire length of the esophagus. However, in about one fifth of the experiments stimulation of the vagus caused contraction of the esophagus including the lower two thirds or even the entire length of the cervical portion. In these cases the pharyngo-esophageal nerve may cause contraction of only the upper two thirds of the cervical portion on stimulation.

b) *Cat*. In 14 experiments the cervical portion of the esophagus received its motor innervation almost solely from the pharyngo-esophageal nerve. It arises from the vagus nerve just above the nodose ganglion in a short common trunk with the superior pharyngeal nerve. It goes medially towards the pharynx, being almost parallel but dorsal and anterior to the superior laryngeal nerve. As soon as it reaches the pharynx at its dorsolateral aspect it gives branches to the inferior constrictor of the pharynx; then it courses posteriorly dorsal to the terminal branches of the superior laryngeal nerve to lie on the lateral surface of the esophagus where it branches freely. Stimulation of this nerve resulted in immediate tetanic contraction of the cervical portion, which, similar to the dog, is about one third of the entire length of the esophagus.

One interesting variation may be recorded here. It was found in two animals that there was a communicating branch between the superior laryngeal and the pharyngo-esophageal nerves. Results obtained by stimulation of the different portions of these nerves indicated that some motor fibers for the esophagus join the pharyngo-esophageal nerve by way of the superior laryngeal and the communicating branch. Stimulation of the superior laryngeal distal to the communicating branch had no effect on the esophagus—a condition different from that described in the dog.

The effect of stimulation of the peripheral end of the vagus nerve was confined to the thoracic portion of the esophagus. Only occasionally the lowermost inch or so of the cervical portion of the esophagus was involved. The effect of vagal stimulation on the thoracic esophagus was different from that in the dog. The upper thoracic part or the middle third of the esophagus responded by a strong, immediate and sustained contraction, while the lower third showed a sluggish spasmodic contraction of slow onset.

c) *Monkey (Macacus Rhesus)*. The result of the experiments in three monkeys in regard to the innervation of the cervical portion of the esophagus was entirely in accord with Kahn's report. The external branch of the superior laryngeal nerve possesses an esophageal branch, stimulation of which caused strong contraction of the upper three fourths of the cervical portion of the esophagus. The cervical portion of the esophagus in this species of animal is about one fifth of the entire gullet in length. Stimulation of the pharyngeal branch of the vagus caused contraction of the pharyngeal muscles, the esophagus not being involved. Stimulation of the peripheral end of the vagus caused contraction of the entire length of the esophagus. It is interesting to record here that in response to vagal stimulation the middle third of the esophagus showed an immediate tetanic contraction followed after a period of short latency by further, but sluggish, contraction. This latter occurred simultaneously with, and similar in character to, the contraction of the lower third during

the stimulation. Apparently these findings could be explained on the basis of the known muscular constituents of the esophagus of this species.

d) *Rabbit*. In the first two experiments it was found that stimulation of the superior laryngeal nerve caused no contraction of the esophagus. No nerve was present in the same anatomical position as the pharyngo-esophageal nerve in the dog or cat. Stimulation of the peripheral end of the vagus in the neck caused strong contraction of the entire length of the esophagus. This was in accord with all the previous reports (15, 16b, 17) and there has been no controversy in this species of animal. In his experiments in the rabbit, Reid (15) stated that: "When the vagi are cut in the neck, even as high as the origin of the superior laryngeals, the pharynx and a very small portion of the esophagus next to it, still retain their healthy action, for, as we have already seen, these receive their motor filaments from the pharyngeal branches of this nerve." However, apparently he based this statement upon his observation in the dog and in neither case did he dissect out the special branch to the esophagus.

In later experiments we found that on stimulation of the central end of the vagus nerve a reflex contraction of the upper end of the esophagus was obtained, and was not affected after section of the other vagus nerve in the neck and section of the superior laryngeal nerve on both sides. Judging from the results of the study of the reflex contraction of the esophagus on stimulation of the central end of the vagus in the dog, which will soon be described in this communication, we were led to look for some additional nerve supply to the upper end of the esophagus other than the vagus. On careful dissection an esophageal branch was found given off from the pharyngeal branch of the vagus but was hidden underneath the pharyngeal muscles on both sides. Stimulation of this branch caused contraction of the upper half of the cervical portion of the esophagus, while section of this branch homolateral to the vagus being stimulated at the central end greatly diminished the reflex response of the esophagus, and subsequent section of the same branch on the other side abolished the reflex esophageal contraction completely. Similar results were obtained in three rabbits.

e) *Guinea pig and albino rat*. In both of these species the upper half of the cervical portion of the esophagus receives its innervation mainly from the external branch of the superior laryngeal nerve, as evidenced by its marked contraction on stimulation of the nerve in three animals of each species. Stimulation of the peripheral end of the vagus caused contraction of the entire length of the esophagus but that of the upper third of the cervical portion was much weaker.

Thus, it is seen that a separate pharyngo-esophageal nerve was found in the dog and cat and a special esophageal branch in the other species of animals studied. Judging from the effect on the cervical portion of the esophagus on faradic stimulation, there seems to be little doubt about their physiological action, which, however, is further supported by the following experiments in the dog and cat.

## 2. *Experimental Evidences That the Pharyngo-esophageal Nerve is the Main Motor Nerve Supply to the Cervical Portion of the Esophagus*

a) *Acute experiments*. The effect of faradic stimulation of the pharyngo-esophageal nerve on the cervical portion of the esophagus has been described above.

Stimulation of this nerve and the peripheral end of the vagus at the same time always gave a strong tetanic contraction of the entire length of the esophagus. When the esophagus including the recurrent laryngeal nerves was transected at the base of the neck and the cervical portion was then freed from all the adjacent connections, stimulation of the pharyngo-esophageal nerve demonstrated very clearly the simultaneous contraction of the longitudinal and circular muscular layers, as shown by the simultaneous shortening and constriction of the tube.

In some experiments in the dog, when a balloon inflated with about 20 cc. of air was placed in the esophagus at the junction of the cervical and thoracic portions, it could be pushed up into the cervical portion by stimulation of the peripheral end of the vagus nerve or down into the thoracic portion by stimulation of the pharyngo-esophageal nerve, evidently due to the strong tetanic contraction of the different portions of the tube.

When the cervical portion of the esophagus was freely exposed and the central end of the superior laryngeal nerve stimulated under light ether anesthesia, reflex swallowing was easily induced in the dog and followed by a distinct peristaltic wave. The wave passing along the thoracic portion could be felt by putting a finger into the lower esophagus through gastrostomy. The effect of sectioning the vagi in the middle of the neck or the pharyngo-esophageal nerves on the peristaltic movements of the esophagus was thus easily determined. Six experiments were done and results were consistent. Bilateral vagotomy did not affect the peristalsis over the cervical portion of the esophagus but abolished that over the thoracic. Subsequent section of the pharyngo-esophageal nerve on both sides made the whole tube quiescent. When this nerve was cut on both sides before the vagi, there was no more peristalsis over the cervical but still over the thoracic portion of the esophagus. Unilateral section of the nerve has no apparent effect on the peristalsis.

b) *Chronic experiments.* In order to ascertain the rôle of the pharyngo-esophageal nerve in the normal animal, this nerve was cut aseptically on both sides just above the level of the cricoid cartilage under local or general anesthesia in two dogs and three cats. In all animals similar symptoms of dysphagia and regurgitation developed. In some animals coughing occurred very often during a meal.

By roentgenological examination with a barium meal in the dog, paralysis of the cervical portion of the esophagus with retention of the meal was revealed, although the thoracic portion of the esophagus was functionally normal. Balloon examinations also checked very well with this finding (fig. 1c). Operation in the same anatomical region but cutting the superior laryngeal nerve instead of the pharyngo-esophageal resulted in no apparent disturbance of deglutition.

Roentgenological examination in the cat showed that the paralysis involved the whole cervical portion as well as the adjacent upper fourth of the thoracic portion of the esophagus (fig. 1d), while the portion below was active.

In the course of from three to five weeks each animal with the pharyngo-esophageal nerve sectioned became free from all these symptoms and roentgenological examination was negative except in the dog, which still showed slight retention of meal in the upper end of the cervical portion of the esophagus two and a half months or longer after the operation (fig. 1g).

In order to further evaluate the function of the pharyngo-esophageal nerve in the cat, one experiment was done in which the vagus nerves were sectioned aseptically

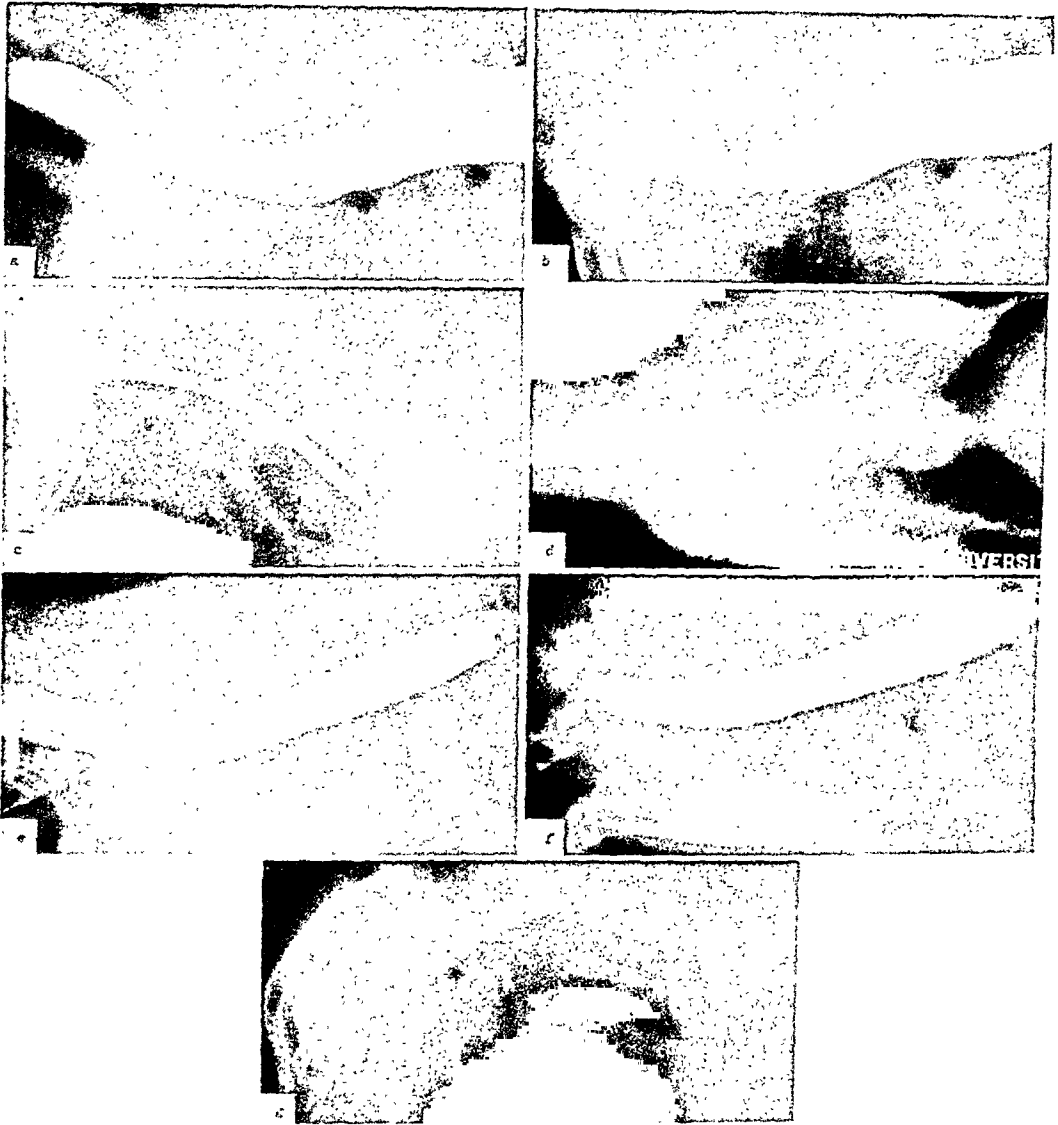


Fig. 1a. ROENTGENOGRAPH showing the condition of the esophagus in a dog after bilateral vagotomy at the level just posterior to the larynx. Milk containing barium sulfate has been given to the animal. *b*. Showing the same animal as in *a*. The roentgenograph was taken during deglutition. The peristaltic function of the cervical portion of the esophagus was preserved but the part below was paralyzed. *c*. Retention of a balloon in the paralyzed cervical portion of the esophagus after bilateral section of the pharyngo-esophageal nerve in a dog. *d*. Retention of barium meal in the cervical portion and the upper fourth of the thoracic portion of the esophagus after bilateral section of the pharyngo-esophageal nerve in a cat. *e*. Appearance of the esophagus after a barium meal in a cat after bilateral vagotomy in the neck posterior to the level of the larynx. Tracheotomy was also performed. *f*. Roentgenograph taken during deglutition of the same cat as shown in *e*. The cervical portion of the esophagus still possessed peristaltic activity. *g*. Retention of barium meal in the upper third of the cervical portion of the esophagus in a dog two and a half months after bilateral section of the pharyngo-esophageal nerve.

in the neck posterior to the level of the larynx together with tracheotomy under local anesthesia. Subsequent barium meal examinations revealed peristaltic function confined to the cervical portion of the esophagus (fig. 1e, f). Paralysis of the thoracic

portion of the esophagus was complete and no tertiary peristalsis appeared in a period of 10 hours after the operation.

### 3. *Reflex Spasmodic Contraction of the Esophagus on Stimulation of the Central End of the Vagus Nerve*

Meltzer and Auer (35) reported that a reflex tetanic contraction of the esophagus on stimulation of the central end of the vagus nerve is quite a reliable finding in the dog. When weak stimulation is used the tetanic contraction is confined to the cervical portion of the esophagus, but with a much stronger stimulation the thoracic portion also may be involved. They did not study the efferent pathway involved in this reflex, and no confirmation of their work was reported.

In the course of the present investigation with light ether or nembutal anesthesia we demonstrated in dogs consistently that there was a reflex tetanic contraction of the lower part of the pharynx and the cervical portion of the esophagus on stimulation of the central end of the vagus nerve. There was usually a period of latency of from two to four seconds, and the onset of the tetanic contraction was often preceded by some irregular twitchings. As soon as the stimulation stopped there was immediate relaxation. Subsequent stimulation of the same nerve gave the same response, but the period of latency was often much reduced.

It was consistently found that section of the vagus of the opposite side has no effect on this reflex. Except in one case this reflex was always abolished by section of the pharyngo-esophageal nerve of the same side. In these cases stimulation of the central end of the vagus nerve of the opposite side again produced the same phenomenon but also was abolished by section of the pharyngo-esophageal nerve of that side. In the exceptional case the reflex was abolished only by bilateral section of the nerve.

### 4. *Periodic Spasm of the Esophagus During Gasping*

In one dog under ether anesthesia after the chest was opened with artificial respiration, gasping type of respiration developed during the course of the experiment without apparent reason. At the same time there was a periodic spasmodic contraction of the cervical portion of the esophagus corresponding to the later part of the inspiratory phase of the gasping. This spasmodic contraction disappeared after the spontaneous cessation of the gasping.

In subsequent experiments efforts were made to reproduce the same phenomena. Gasping type of respiration was regularly produced in 8 dogs several minutes after ligation of the common carotid and the vertebral arteries on both sides at the base of the neck and was always associated with the periodic esophageal spasm during the later part of the inspiration. The strength of the spasm apparently correlated with the degree of the gasping.

In one experiment spasm of the esophagus thus produced was found involving the entire length of the esophagus, as determined by putting a finger into the lower thoracic esophagus through the cardia. In another one the same thing was found by direct observation after the chest was opened. In the rest of the experiments, whether or not the thoracic portion of the esophagus was involved was not determined.

In the experiments described above section of both vagus nerves in the neck



abolished the spasm of the entire thoracic portion of the esophagus but had no effect on that of the cervical portion. The latter was abolished by bilateral section of the pharyngo-esophageal nerve in all the experiments.

#### DISCUSSION

Review of the literature indicates that the interesting reports of the earlier workers (16-18) concerning the innervation of the cervical portion of the esophagus have been entirely ignored by the later investigators. As a matter of fact these old but fundamental conceptions regarding the neuromuscular mechanism of this organ are not taught to students of physiology, who, on the other hand, are always impressed by the traditional teaching that this part of the organ is controlled by the recurrent laryngeal branch of the vagus nerve regardless of the species. This classical conception is apparently based mainly on the experiments in the rabbit, which have given consistent results probably ever since the time of Reid (15), and has been generalized to all the mammals, among which the dog and the cat have been the commonly used experimental animals. Not only the recent workers (22-27, 31) have supported the present conception, but also the earlier investigator, Meltzer, who made brilliant studies on the peristaltic activities of the esophagus, apparently had the same idea. During his experiments of transecting the esophagus in the dog (36, p. 268), he emphasized that the recurrent laryngeal nerves should not be injured, and mentioned no nerve supply from other sources.

The present investigation among the different species of the ordinarily used laboratory animals, however, strongly supports the overlooked old idea that the cervical portion of the esophagus does receive some motor innervation in addition to the recurrent. There is no exception even in the rabbit to the general finding that the cervical portion of the esophagus receives a double innervation from widely separated vagal branches, although there are species variations in regard to their anatomical course and extent of distribution.

Evidence has been presented in both acute and chronic experiments in the dog proving that the pharyngo-esophageal nerve is the motor nerve that controls all the following activities of the cervical portion of the esophagus. These activities include peristalsis, the reflex tetanic contraction on stimulation of the central end of the vagus nerve and the periodic spasm during gasping as observed in the acute experiments. Practically all these activities are abolished by bilateral section of this nerve. In chronic experiments in both the dog and cat, the cervical portion of the esophagus was not paralyzed by section of the vagi just posterior to the level of the larynx but by bilateral section of this pharyngo-esophageal nerve, although the permanent paralysis is limited only to the very upper portion in the dog. A diagram has been constructed to illustrate the motor innervation of the esophagus of the dog (fig. 2).

The question arises as to why such an important nerve has not drawn the due attention of recent investigators in this field. The old literature could have been overlooked, but the generalization of the manner of the innervation in the rabbit is not likely to be the only answer to the question of the origin of the current conception which, as pointed out in the review of the literature, has received much experimental

support purporting to prove that the cervical esophagus is innervated by the recurrent branch of the vagus.

There are several reasons that might account for the discrepancies. In the first place there could be such exceptional cases in the dog as noted in some of the present experiments, in which the entire cervical portion of the esophagus may receive motor innervation from the recurrent branch of the vagus in addition to the pharyngo-esophageal nerve. In such a case further efforts to search for an additional

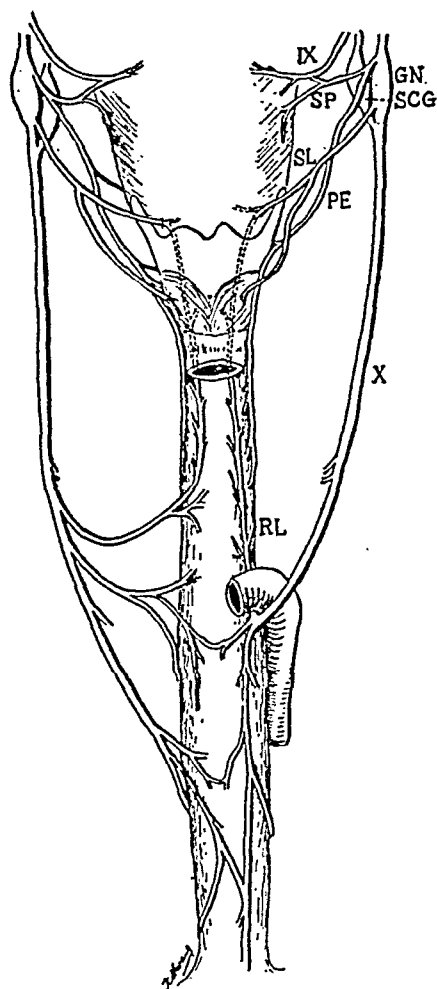


Fig. 2. DIAGRAM ILLUSTRATING THE INNERVATION of the esophagus in the dog. GN: ganglion nodosum; PE: pharyngo-esophageal nerve; RL: recurrent laryngeal nerve; SCG: superior cervical sympathetic ganglion; SL: superior laryngeal nerve; SP: superior pharyngeal nerve; IX: glossopharyngeal nerve; X: vagus nerve.

motor nerve are apparently not indicated. The manner of innervation of the esophagus in the rabbit is another good example.

In the second place, the method of the observation is very important in the interpretation of the results. Apparently several different methods have been used by these recent workers. By direct inspection Inaoka (22) observed that peripheral vagal stimulation does not act on the cervical esophagus, which is only pulled down passively. But direct stimulation of the recurrent nerve results in contraction of the cervical esophagus. The failure of the vagal stimulation, he believes, is due to a block at the inferior cervical ganglion. This is not in accord with our knowledge of anatomy and we did not record similar results. The results of stimulation of the

vagus stump and of the recurrent laryngeal usually correlated very well when care was taken to avoid the spread of the current.

According to some investigators (23, 27) records of contraction of the cervical esophagus upon vagal stimulation have been obtained by means of a recording tambour connected to a balloon placed in the cervical portion of the esophagus. In this case it is not impossible that a purely mechanical traction of the cervical portion of the esophagus by the shortening of the thoracic portion on vagal stimulation may cause a rise of the pressure in the balloon simulating contraction, or the contraction of the lower part of the cervical esophagus could be interpreted as contraction of the entire cervical portion.

In chronic experiments the roentgenoscopic method has often been used (1, 26). Unless the deglutition movement is carefully observed as shown in figure 1b, f, roentgenographs such as figure 1a, e, may give rise to the impression of paralysis of the entire esophagus. Those who make vagotomy in the neck usually leave the right recurrent laryngeal nerve intact (20, 27), believing that the innervation of the cervical portion of the esophagus is preserved in this way. Jurica (26) reported complete paralysis of the striated portion of the esophagus after he made successful double vagotomy at the middle of the neck in the cat. However, examination of the roentgenographs in his article reveals no retained meal in the cervical esophagus, a fact which could be due to the preserved motor innervation by the pharyngo-esophageal nerve as shown in the present experimentation.

One conception that might lead people to overlook the activity of the cervical portion of the esophagus is the effect of the squirting action by the rapid contraction of the muscles of the mouth. By this action liquid and semisolids are squirted down the esophagus instead of being moved by peristalsis. This is proposed by Kronecker and Meltzer (37) and supported by Cannon (38) and Meltzer (39). The latter demonstrated a dog drinking in a perfectly normal manner after the muscularis of the entire cervical esophagus has been removed. This is not in accord with the present report, for dysphagia, although temporary, did develop after cutting the pharyngo-esophageal nerves, and also retention of food (milk and barium sulfate) was found in the cervical portion of the esophagus (fig. 1g). This retained food detected by the roentgenologic method could not be explained simply by the separated barium sulfate stuck to the esophageal wall as suggested by Meltzer (39), since we have never succeeded in causing barium sulfate to stick to the esophageal wall of the normal dog by using different food mixtures, liquid or solid. The copious secretion of the esophageal glands of this animal may prevent adherence of the barium. Judging from these points we can say that the 'squirting' action is not the only mechanism by which liquid meals pass through the cervical portion of the esophagus and that for completing the process of deglutition, normal peristaltic activities of this portion of the organ cannot be neglected.

The immediate paralysis of the cervical portion of the esophagus following bilateral section of the pharyngo-esophageal nerve in the dog and cat signifies the important rôle played by this nerve in normal physiological function. The discrepancy that the length of the paralyzed portion of the esophagus following bilateral section of the pharyngo-esophageal nerve is longer than what is expected on the basis

of stimulation of this nerve in acute experiments in the cat is not readily understood. Although there is complete functional recovery in the course of from three to five weeks, residual signs in the dog could still be found two to four or more months later. This functional recovery of the once paralyzed organ cannot be explained by regeneration of the nerve since, on the one hand, a period of three or five weeks is too short for the possible regeneration, and on the other hand, the recovery is not complete at the uppermost portion in the dog. Further investigation is being carried on to find the correct explanation, but in all probability the function is taken over by the recurrent laryngeal nerves. This assumption is based upon the fact that the recovery is more complete over the lower part of the cervical portion of the esophagus, which is often found to be innervated by both the pharyngo-esophageal and the recurrent laryngeal nerves. Should this be true, the significance of the double innervation of this organ is evident.

As pointed out before, the anatomical course of the pharyngo-esophageal nerve, as determined by dissection and electric stimulation in the dog and cat, is variable. In general it is well in accord with what is described by Espezel and Kahn. Regarding its origin, we agree with Kahn, that is, it originates from the vagus in a common trunk with the superior pharyngeal nerve and not from the superior cervical sympathetic ganglion as claimed by Espezel. However, its close relationship to this ganglion, as described before, is noticeable. Probably the finding of stasis of food in the upper esophagus after extirpation of this ganglion in the dog, as reported by Kure and others (32, 33), could be explained on the basis of incidental resection of that portion of the nerve which is bound to the ganglion.

The mechanism of the periodic spasmodic contraction of the esophagus during gasping in the dog certainly deserves further investigation. The efferent pathways are apparently the vagus nerve for the thoracic portion and the pharyngo-esophageal for the cervical. Since this spasmodic contraction occurs in the inspiratory phase and its strength is usually correlated with the extent of the gasping, it seems rational to assume that the esophageal musculature, at least the thoracic portion, could be considered as one of the accessory respiratory muscles.

Regarding the innervation of the human esophagus little more is known than the classical conception. Since there are wide variations among different mammals it is difficult to predict to which species the manner of innervation of the human esophagus might correspond. No nerve corresponding to the pharyngo-esophageal has been described in the human being, nor any esophageal branches from the superior laryngeal. However, clinical reports of 'cardiospasm' with paralysis of the lower two thirds of the esophagus (40) and 'dysphagia' due to paralysis of the upper third of the esophagus (41) should call our attention to the possibility of separate innervation of the different portions of the human esophagus.

#### SUMMARY

The innervation of the esophagus has been studied in the dog, cat, rabbit, monkey, guinea pig and rat. There is no exception to the general conclusion that the cervical portion of the esophagus receives double innervation from widely separate vagal branches. Species differences regarding the anatomical course and the extent

of distribution have been described and discrepancies of opinions among different authors according to the literature have been discussed.

The main motor innervation of the cervical portion of the esophagus in the cat and dog is the pharyngo-esophageal nerve, which arises from the vagus above the ganglion of nodosum. Stimulation of this nerve in these animals causes strong tetanic contraction of the cervical portion of the esophagus.

In chronic experiments in the dog and cat, bilateral section of the vagus trunk alone resulted in paralysis of the whole esophagus except the cervical portion. After bilateral section of the pharyngo-esophageal nerve alone there was temporary paralysis of the entire cervical esophagus accompanied by symptoms of dysphagia. Apparent functional recovery was complete in three to five weeks.

In acute experiments in the dog, that the pharyngo-esophageal nerve is the main efferent nerve to the cervical portion of the esophagus is further evidenced by the abolition of all of its activities after section of this nerve. The activities of this portion of the esophagus here include the peristaltic movements, the reflex tetanic contraction on stimulation of the central end of the vagus nerve, and the periodic spasm during gasping.

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# EFFECT OF SECRETIN AND PANCREOZYMIN ON AMYLASE AND ALKALINE PHOSPHATASE SECRETION BY THE PANCREAS IN DOGS

C. C. WANG, M. I. GROSSMAN AND A. C. IVY

*From the Department of Clinical Science, University of Illinois College of Medicine*

CHICAGO, ILLINOIS

**T**WO hormones extractable from the upper intestinal mucosa, secretin and pancreozymin, are concerned in the regulation of pancreatic secretion. This study was undertaken in order to obtain further information on the rôle of each of these hormones in the control of enzyme secretion by the pancreas.

Previous work has shown that secretin acts as a stimulus mainly for water and bicarbonate secretion by the pancreas. Thus, the pancreatic juice secreted in response to it is relatively poor in enzymes when compared with either pancreozymin plus secretin stimulation or stimulation by parasympathomimetic drugs. By contrast, pancreozymin (10-12) stimulates the secretion of enzymes (amylase, lipase and trypsinogen) without influencing the rate of water secretion, that is without changing the volume of juice put out per minute.

While it is apparent that secretin does not evoke a juice rich in enzymes, there are discrepancies in the literature concerning whether it has any stimulating action on enzyme secretion (1-9). Some of these discrepancies can probably be explained by the presence of varying amounts of pancreozymin in the secretin preparations that were tested. Therefore, the first question which we set out to answer was, does an increase in the amount of circulating secretin increase significantly the total output of amylase in the pancreatic juice per unit of time?

Another question that it was considered desirable to investigate pertained to the secretion of alkaline phosphatase in the pancreatic juice of the dog (13,14). Histochemically phosphatase occurs only in the cells lining the ductules and is absent from the acinar cells (15-17). This is illustrated in figure 1, a photomicrograph of a section of dog's pancreas stained by the Gomori technique for alkaline phosphatase (15). The effect of secretin and pancreozymin on the secretion of alkaline phosphatase has not been studied previously.

## METHODS

Acute experiments were carried out on dogs which were anesthetized with an intravenous injection of pentobarbital (32 mg/kg. body weight). Prolonged anesthesia was maintained when necessary by additional intravenous injections. The last feeding of the animals was 24 hours before the experiment. Cannulae were inserted into the left femoral vein for secretin infusion and also into the trachea.

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Received for publication June 28, 1948.

To preclude stimulation of the pancreas by bile or acid in the duodenum, the common bile duct and the pylorus were occluded. Pancreatic juice was collected by a cannula inserted into the major pancreatic duct.

Highly purified secretin dissolved in saline was continuously injected intravenously by means of a perfusion pump into the left femoral vein of the animal. Three different secretin preparations were used. All were devoid of vasodepressor substances. Secretin *A* was about twenty times as potent as SI (11); secretin *B*, twelve times; and secretin *C*, eight times<sup>1</sup>. The pancreozymin used in the present work was prepared by the aniline precipitation procedure (11) and also showed no vasodepressor effect.

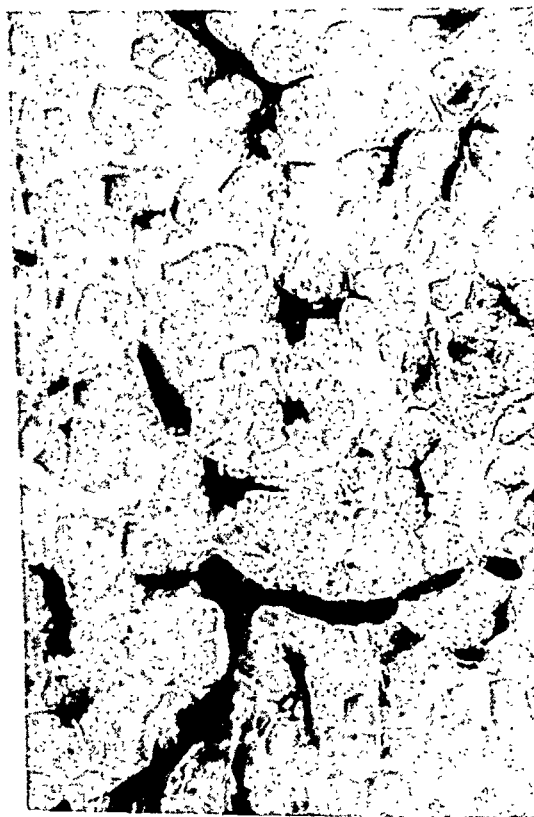


Fig. 1. PHOTOMICROGRAPH OF DOG'S PANCREAS stained for alkaline phosphatase by the method of Gomori. Only the ductule cells stain positively.

The first injection rate was adjusted so as to maintain a rate of flow of juice of about 1.5 to 3.0 cc. in 15 to 30 minutes. Increase of flow was brought about by increasing the rate of injection of secretin. In order to clear out the initial concentrated juice accumulated in the pancreatic duct system, in the cannula and in the connecting tube and, also, owing to the fact that animals were usually more sensitive to the secretin stimulation in the beginning, the juice collected during the first hour of response to secretin was discarded. Pancreozymin was injected in a single dose of 20 mg. of the preparation employed during the response to a constant dose of secretin. The pancreatic juice was collected in 15 to 30 minute portions, in graduated centrifuge tubes on ice, and was stored in the freezing unit of the refrigerator overnight. At least three or four samples were collected at each secretory rate level. All samples were assayed for amylase and alkaline phosphatase content.

The amylase content of successive samples of juice was measured by the modified Willstatter method described in detail by Schmidt, Greengard and Ivy (18). The amylolytic activity unit was expressed in mg. of maltose per cc. of juice as derived from the thiosulphate titration. From the amylase content of the various samples, the minute output of this enzyme was calculated by multiplying the concentration in mg/cc. by the volume of the sample in cc. divided by the duration of the collection in minutes.

<sup>1</sup> These secretin preparations were kindly supplied by Dr. E. D. Campbell of the Lilly Research Laboratories, Indianapolis, Ind.



According to the generally accepted conception that the three principal enzymes of the pancreatic juice, namely the amylase, trypsinogen and lipase, are parallel in their concentration (9, 10, 11, 20, 21), reliance has been placed on the estimation of amylase as an index of the concentration changes of the three principal enzymes of the juice under the experimental conditions. The method for amylase determination was simpler and gave much greater sensitivity than could be obtained for the other two enzymes.

The content of alkaline phosphatase of the juice was estimated essentially by the method described by Shinowara, Jones and Reinhart (19). The final  $pH$  of the reaction mixture of substrate and pancreatic juice is  $9.3 \pm 0.15$  at  $37^\circ C$ . The method for the estimation of the 'initial' inorganic phosphate of juice and of the 'total' inorganic phosphate of juice after incubation was devised by Dr. J. Canepa of this laboratory after Holman's method for inorganic phosphate. The latter method is outlined briefly as follows: To 1 cc. of 'total' phosphate protein-free fluid, in a 15 cc. graduated conical centrifuge tube, water is added to make the total volume 5 cc. At the same time a 'blank' is prepared by using 1 cc. of 'blank fluid' instead of 'total' phosphate protein-free fluid. 'Blank fluid' is prepared similarly to the 'total' phosphate protein-free fluid by using water instead of pancreatic juice before incubation. To all the tubes add in succession 1 cc. of 10 N  $H_2SO_4$ , 1 cc. of 2.5 per cent ammonium molybdate and 1 cc. of 20 per cent KI (containing 0.5 per cent  $Na_2CO_3$ ). Mix by tapping after each addition. Put into a boiling water-bath for exactly 15 minutes. Cool. Next add 0.4 cc. of 0.25 per cent anhydrous sodium sulphite. Bring the final volume to 10 cc. or 15 cc. depending upon the depth of the color.

The density of the color developed by the reduction of phosphomolybdic acid is read on a Coleman spectrophotometer at 670  $m\mu$ . From the readings, the amount of 'total' phosphate can be estimated by reference to a calibration curve constructed by using solutions containing known amounts of phosphate. The 'initial' inorganic phosphate of pancreatic juice is determined in the same way by using an 'initial' protein-free fluid instead of 'total' phosphate protein-free fluid. A 'blank' is prepared similarly to the preparation of 'initial' protein-free fluid by using water instead of pancreatic juice.

The 'total' inorganic phosphate in mg. of P per 100 cc. of juice after incubation minus the 'initial' inorganic phosphate in mg. of P per 100 cc. of juice without incubation equals the liberated inorganic phosphate. The alkaline phosphatase activity unit is therefore expressed in mg. of phosphorus per 100 cc. of pancreatic juice liberated from the incubation.

From the alkaline phosphatase contents of the various samples, the minute output of this enzyme was calculated by multiplying the concentration by the volume of the sample in cc. divided first by 100 and again by the time of collection of the sample in minutes.

## RESULTS

In order first to show the effect of constant administration of secretin on the amylase and alkaline phosphatase content of the pancreatic juice over an experimental period, control experiments were carried out (table 1). The volume, amylase concentration and alkaline phosphatase concentration of the pancreatic juice, during constant administration of secretin over a period of three hours, is shown in the experiment depicted in figure 2. This experiment, begun after an hour's previous secretin stimulation, clearly demonstrates the general tendency of changes of the juice.

The volume usually showed a gradual increase and finally a plateau was reached. The amylase content showed little fluctuations, but generally there was a tendency to decline slowly toward the last phase of the experiment. A clear fall of the con-

centration, however, was observed in the case of alkaline phosphatase. Calculations (table 1) show that the minute output of amylase remained essentially unchanged while that of the alkaline phosphatase shows a slowly diminishing rate.

As regards the sensitivity of different animals to the effect of secretin, considerable variation was observed. This sensitivity can be judged from the rate of spontaneous flow of juice before the application of secretin stimulation and also from the

TABLE 1. FLUCTUATIONS IN VOLUME AND IN CONCENTRATION AND MINUTE OUTPUT OF ALKALINE PHOSPHATASE AND AMYLASE OF PANCREATIC JUICE DURING CONSTANT SECRETIN ADMINISTRATION (2 DOGS)

	DOG NO.	PORTION NUMBER											
		1	2	3	4	5	6	7	8	9	10	11	12
Volume (cc.)	2	3.2	3.1	3.3	3.6	3.5	4.1	4.0	3.7	3.9	4.0	3.9	4.0
	8	2.8	3.2	2.9	4.0	4.5	4.7	4.1	4.5	5.0	5.0	5.0	
Amylase conc.	2	652	412	360	400	360	340	320	310	359	292	274	295
	8	473	442	414	391	380	410	476	313	341	346	330	
Amylase min. output	2	104.1	63.9	59.4	76.5	63.0	69.7	64.0	57.4	70.1	58.4	52.2	59.0
	8	88.0	94.0	80.0	104.0	114.0	128.5	130.0	93.3	114.0	115.0	110.0	
Alk. phosphatase conc.	2	3.00	3.40	3.00	1.70	2.40	2.00	2.40	2.00	1.60	1.80	1.20	1.00
	8	2.10	1.45	1.40	1.50	1.40	0.95	0.75	0.80	0.70	0.85	0.65	
Alk. phosphatase min. output	2	0.0048	0.0053	0.0050	0.0031	0.0042	0.0041	0.0048	0.0037	0.0031	0.0036	0.0023	0.0020
	8	0.0034	0.0031	0.0026	0.0040	0.0042	0.0030	0.0021	0.0024	0.0023	0.0028	0.0022	

Dog 2, 9 kg. Secretin B: 3.4 mg/20 min.; samples collected in 20-min. portions.

Dog 8, 10 kg. Secretin A: 1.2 mg/15 min.; samples collected in 15-min. portions.

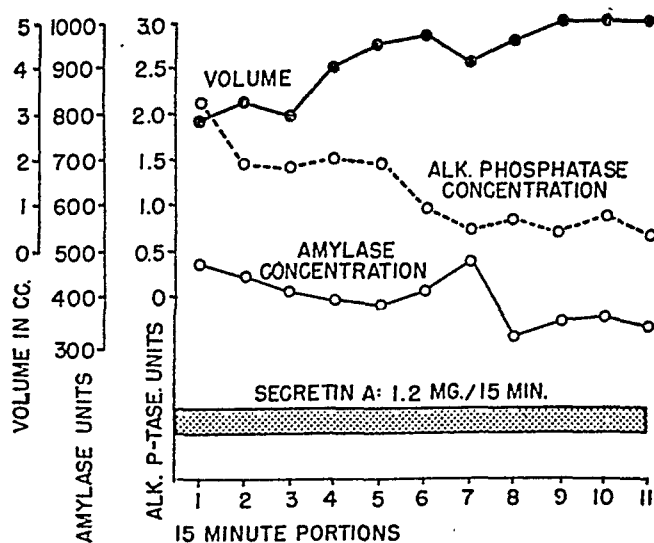


Fig. 2. Dog 8, 10 kg. Fluctuations in volume and in concentration of amylase and alkaline phosphatase of pancreatic juice during constant secretin administration

initial response of the gland to the first rate of secretin administration. Moreover, the sensitivity of the same animal to the effect of secretin is by no means constant. Generally speaking, they are more sensitive in the initial phase of secretin stimulation, then gradually become stabilized and maintain the condition for three or four hours or sometimes longer. It was for this reason that a previous secretin stimulation, of approximately one hour before the collection of samples, was necessary.

However, a phenomenon of exhaustion might be observed after an especially prolonged maximal stimulation over a period of one to two hours. In both the experiments shown in table 1, a moderate intensity of secretin stimulation was maintained throughout the whole period of over three hours. It was in this period that the most stable results were obtained.

The question as to whether or not secretin itself has any stimulating effect on the enzyme production of the pancreas has been answered in the results obtained from seven experiments. The volume, enzyme concentration and enzyme minute output of the pancreatic juice obtained before and after the increase of secretin stimulus are shown in table 2. Essentially similar results are obtained in different animals either with the secretin *A* or the secretin *B*. Figure 3 drawn from the observations on *dog 12* serves as a typical illustration. Portions 1 to 4 (inclusive) were taken as controls during a constant rate of perfusion of secretin. The variation in the volume of juice was small. The amylase concentration and minute output also showed only small fluctuations. During the period represented by portions 5 to 7, the secretin stimulus was quadrupled, and the result was a sharp rise in volume soon reaching a plateau; concomitantly a great fall in amylase concentration occurred. The minute output of the enzyme, however, remained essentially unchanged. Although portion 5 did show an 8 per cent increase as compared with the previous one (portion 4), it could hardly be attributed to an increase of enzyme secretion under stimulation by secretin since as much as 38 per cent fluctuation of enzyme output occurred among the control portions 1 to 4. Moreover, this rather insignificant increase of the enzyme output might be attributed to a 'washing out' from the gland of the enzyme which was presumably accumulated in the glandular passages of acini which were inactive before the increase of secretin stimulation took effect, the process therefore being a passive one. This 'washing out' assumption is strongly supported by the evidence that the amount of the enzyme discharged in the portions 6 and 7, though still under an increased rate of secretin injection, did not show a continued increase following the immediate increase in portion 5.

In the experiment as represented in figure 3, the initial level of secretin injection rate (portions 1-4) was very low and the secretin stimulation was presumably not much above threshold, whereas in the second phase represented in portions 5 to 7 the secretin stimulus was definitely maximal. The fact that secretin has no stimulating effect on enzyme production of the pancreas is quite evident. Nor could this be attributed to exhaustion of the gland, since in some experiments, pancreozymin injected at the last phase of maximal secretin stimulation has never failed to cause an abundant increase in amylase concentration in the juice.

In order to demonstrate the effect of secretin on the alkaline phosphatase concentration and its minute output in the *dog's* pancreatic juice the same procedure has been used as with amylase. The volume, alkaline phosphatase content and minute output of the juice obtained before and after the increase of secretin stimulus in five experiments are shown in table 3. The observation from *dog 3* is illustrated in figure 4. Portions 1 to 4 (inclusive) were collected under constant secretin stimulation (3.4 mg/20 min.). For portions 5 and 6 the secretin stimulus was almost doubled and this resulted in a sharp rise in volume and a sharp fall in alkaline phosphatase

concentration. A more striking effect was observed by further increase of secretin injection in portions 7 and 8 (8 mg/20 min.). As can be seen from figure 4, the minute output of alkaline phosphatase showed a fall. This continued fall in alkaline

TABLE 2. EFFECT OF SECRETIN ON CONCENTRATION AND MINUTE OUTPUT OF AMYLASE (7 DOGS)

	DOG NO.	PORTION NUMBER									
		1	2	3	4	Increase of Secretin Injection Rate (see below)	5	6	7	8	9
Volume (cc.)	3	2.8	3.7	4.2	4.5		7.4	8.2	9.2 <sup>1</sup>	9.8 <sup>1</sup>	7.5 <sup>1</sup>
	4		2.1	3.0	4.3		6.8	6.5	6.8	7.0 <sup>1</sup>	
	8	4.5	5.0	5.0	5.0		6.9	6.5	6.9		
	10	2.6	3.5	4.1	4.1		12.9	14.8	14.0		
	11	0.9	0.9	1.6	1.9		4.1	4.6	5.5		
	12	1.5	1.4	1.4	2.3		6.1	8.7	8.8		
	13		3.1	3.0	4.3		8.0	8.4	8.8		
Amylase concentration	3	391	349	402	326	217	178	180 <sup>1</sup>	158 <sup>1</sup>	130 <sup>1</sup>	
	4		770	352	250	190	157	162	148 <sup>1</sup>		
	8	313	341	346	330	138	199	152			
	10	1110	735	638	582	243	200	187			
	11	1261	1037	867	604	348	221	230			
	12	939	905	1118	761	310	200	174			
	13		417	540	510	319	270	205			
Amylase min. output	3	54.8	64.6	84.3	73.4	80.2	73.4	83.3 <sup>1</sup>	77.2 <sup>1</sup>	35.0 <sup>1</sup>	
	4		53.9	35.2	35.6	42.8	34.0	36.4	34.5 <sup>1</sup>		
	8	93.3	114.0	115.0	111.0	64.0	86.0	70.0			
	10	192.2	171.5	174.4	159.1	209.0	197.0	173.6			
	11	45.4	33.3	55.5	45.9	54.5	40.7	50.6			
	12	70.5	63.4	78.3	87.5	95.0	86.9	74.7			
	13		86.2	108.0	146.2	170.0	151.2	120.1			

Dog 3, 9.7 kg. Secretin B: 3.4 mg/20 min. during portions 1-4; 6 mg/20 min. during portions 5 & 6; 8 mg/20 min. during portions 7 & 8; samples collected in 20-min. portions.

Dog 4, 11 kg. Secretin B: 6 mg/30 min. during portions 2-4; 12 mg/30 min. during portions 5-7; 15.2 mg/30 min. during portions 8 & 9; samples in 30-min. portions.

Dog 8, 10 kg. Secretin A: 1.2 mg/15 min. during portions 1-4; 4.5 mg/15 min. during portions 5-7; samples in 15-min. portions.

Dog 10, 15.5 kg. Secretin A: 0.65 mg/15 min. during portions 1-4; 2.7 mg/15 min. during portions 5-7; samples in 15-min. portions.

Dog 11, 14.2 kg. Secretin A: 1.1 mg/25 min. during portions 1-4; 4.5 mg/25 min. during portions 5-7; samples in 25-min. portions.

Dog 12, 8 kg. Secretin A: 0.33 mg/20 min. during portions 1-4; 1.47 mg/20 min. during portions 5-7; samples in 20-min. portions.

Dog 13, 9.5 kg. Secretin A: 0.313 mg/15 min. during portions 2-4; 1.38 mg/15 min. during portions 5-7; samples in 15-min. portions.

<sup>1</sup> Second increase of the rate of secretin injection (see above).

phosphatase output is characteristic of such secretin injection experiments, both those in which a constant lower rate of injection is used throughout the whole experiment (table 1) as well as those in which variations in the rate of the secretin adminis-

Fig. 3. Dog 12, 8.0 kg. Effect of secretin on concentration and minute output of amylase. Increase of the rate of secretin injection started after collecting portion 4.

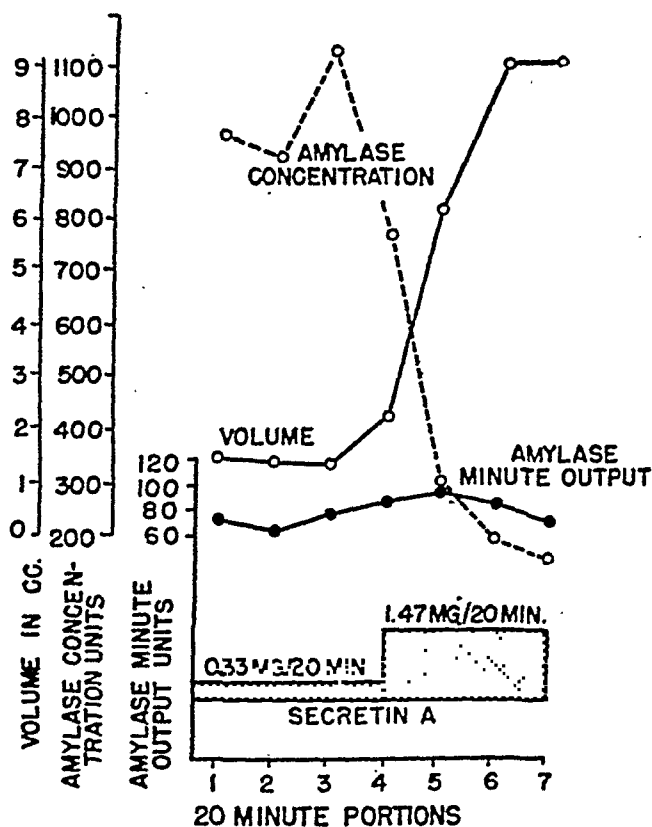


TABLE 3. EFFECT OF SECRETIN ON CONCENTRATION AND MINUTE OUTPUT OF ALKALINE PHOSPHATASE (5 DOGS)

	DOG NO.	PORTION NUMBER								
		1	2	3	4	5	6	7	8	9
Volume (cc.)	3	2.8	3.7	4.2	4.5	7.4	8.2	9.2 <sup>1</sup>	9.8 <sup>1</sup>	
	4		2.1	3.0	4.3	6.8	6.5	6.8	7.0 <sup>1</sup>	7.5 <sup>1</sup>
	8a		2.4	2.4	2.6	3.6	3.6	4.0	4.0	3.6
	8	4.5	5.0	5.0	5.0	6.9	6.5	6.9		
	10	2.6	3.5	4.0	4.1	12.9	14.8	14.0		
Alkaline phosphatase concentration	3	7.90	5.50	4.40	4.70	2.23	1.40	1.30 <sup>1</sup>	0.85 <sup>1</sup>	
	4		6.20	2.90	2.40	2.50	2.20	1.60	1.20 <sup>1</sup>	1.40 <sup>1</sup>
	8a		0.52	1.15	0.89	0.75	0.37	0.37	0.52	0.32
	8	0.80	0.70	0.85	0.65	0.45	0.40	0.35		
	10	18.25	12.10	9.65	7.95	4.24	3.20	3.55		
Alkaline phosphatase min. output	3	0.0111	0.0102	0.0092	0.0101	0.0083	0.0057	0.0061 <sup>1</sup>	0.0042 <sup>1</sup>	
	4		0.0043	0.0029	0.0034	0.0036	0.0050	0.0036	0.0028 <sup>1</sup>	0.0035 <sup>1</sup>
	8a		0.0012	0.0028	0.0023	0.0027	0.0013	0.0015	0.0021	0.0011
	8	0.0024	0.0023	0.0028	0.0022	0.0021	0.0017	0.0016		
	10	0.0316	0.0282	0.0264	0.0217	0.0360	0.0315	0.0330		

Dog 8a, 9 kg. Secretin C: 2.38 mg/10 min. during portions 2-4; 4.75 mg/10 min. during portions 5-9; samples in 10-min. portions.

For dog 3, 4, 8 & 10, see the remarks in table 2.

<sup>1</sup> Second increase of the rate of secretin injection (see above).

tration occur (fig. 4). The fact that secretin does not seem to stimulate the production of alkaline phosphatase in the pancreas was clearly shown. This continued reduction of alkaline phosphatase output following the prolonged administration of secretin might be explained by assuming that the output was not balanced by synthesis.

The results of the experiments with pancreozymin are shown in table 4. All the four experiments were carried out under constant stimulation with secretin. Figure 5 plotted from the results observed on *dog 6* shows the typical changes. As can be seen from the first phase of figure 5 (portions 1-4), both the volume and the amylase concentration showed a constant level. The concentration of alkaline phosphatase, however, fluctuated a little and showed the usual fall. Pancreozymin was injected in a single dose of 20 mg. in the beginning of the second phase (portions

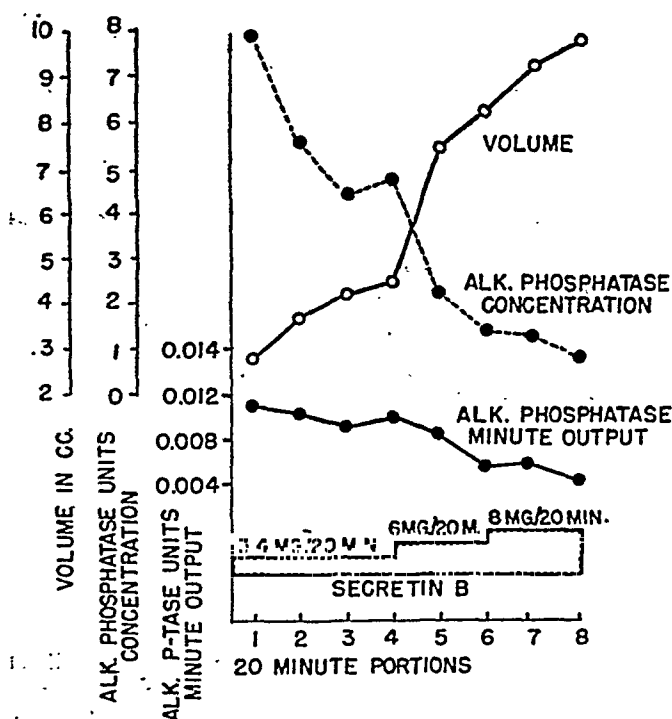


Fig. 4. *Dog 3*, 9.7 kg. Effect of secretin on concentration and minute output of alkaline phosphatase. First increase of the rate of secretin injection started after collecting portion 4. Second increase started after portion 6.

5-8) after collecting portion 4 and again injected in a single dose of 14 mg. in the third phase (portions 9-12) after collecting portion 8. There resulted a three-fold increase of amylase concentration in portion 5 and a more than two-fold increase in portion 9. The concentration of alkaline phosphatase showed a slight decrease. This preparation of pancreozymin did have a slight secretin activity as shown in the rate of flow of juice at portions 5 and 9 in figure 5. However, this slight secretin activity had no confusing effect in these experiments, since the fact that secretin itself has no effect on either amylase or alkaline phosphatase concentration has been repeatedly shown in the experiments already presented.

As can be seen from table 4, the minute output of alkaline phosphatase showed a slowly diminishing fall comparable to that of table 1, in which pancreozymin was not injected. Apparently, pancreozymin has no effect whatever on alkaline phosphatase secretion by the pancreas.

TABLE 4.—EFFECT OF PANCREOZYMIN ON AMYLASE AND ALKALINE PHOSPHATASE (4 DOGS)

	DOG NO.	PORTION NUMBER												
		1	2	3	4	Injection of 20 mg. Pancreozymin	5	6	7	8	9	10	11	12
Volume (cc.)	6	4.0	3.9	4.0	3.9		5.3	3.2	2.6	2.8 <sup>1</sup>	3.5	2.3	3.4	3.6
	7		6.6	7.3	7.0		9.3	7.3	6.8 <sup>1</sup>	8.7	7.0	6.2		
	8	4.6	4.0	3.6	3.6		3.1	2.4	2.4					
	9		4.0	4.0	3.5		4.5	3.9	3.0	3.4	3.0			
Amylase conc.	6	277.8	285.3	291.9	282.5		880.4	280.6	301.3	442.6 <sup>1</sup>	748.6	541.4	337.1	290.0
	7		890.2	883.5	876.1		1163.4	866.6	860.0 <sup>1</sup>	1107.0	965.6	902.4		
	8	152.0	163.0	167.0	148.0		853.0	319.0	226.0					
	9			790.0	790.0		2258.0	900.0	750.0	650.0	790.0			
Amylase min. output	6	111.1	111.2	116.8	110.2		466.6	89.8	78.3	113.9 <sup>1</sup>	262.0	114.5	114.6	104.4
	7		587.5	645.0	613.3		1082.0	632.0	584.8 <sup>1</sup>	963.1	675.9	559.5		
	8	70.0	65.2	60.0	53.3		264.4	76.5	54.2					
	9			316.0	276.5		1016.4	351.0	225.0	221.0	237.0			
Alkaline phosphatase conc.	6	1.46	1.78	1.47	1.15	0.81	1.25	1.67	1.67 <sup>1</sup>	1.20	1.67	0.83	0.94	
	7		3.78	3.36	3.47	2.31	3.15	3.36 <sup>1</sup>	2.94	3.05	3.57			
	8	0.35	0.50	0.35	0.35	0.35	0.30	0.45						
	9		1.90	1.55	1.55	1.35	1.50	1.95	1.30	1.30				
Alkaline phosphatase min. output	6	0.0058	0.0069	0.0059	0.0045	0.0043	0.0040	0.0033	0.0047 <sup>1</sup>	0.0042	0.0038	0.0028	0.0034	
	7		0.0249	0.0243	0.0243	0.0215	0.0230	0.0228 <sup>1</sup>	0.0256	0.0214	0.0221			
	8	0.0016	0.0020	0.0013	0.0013	0.0011	0.0007	0.0011						
	9		0.0076	0.0062	0.0053	0.0061	0.0059	0.0059	0.0044	0.0039				

<sup>1</sup> Dog 6 & 7 received second doses of 14 & 20 mg. pancreozymin, respectively, after this sample.  
Dog 6, 11 kg. Secretin C: 5 mg/10 min. Dog 8, 10 kg. Secretin A: 3 mg/10 min.  
Dog 7, 13 kg. Secretin C: 6.75 mg/10 min. Dog 9, 9 kg. Secretin A: 0.35 mg/10 min.

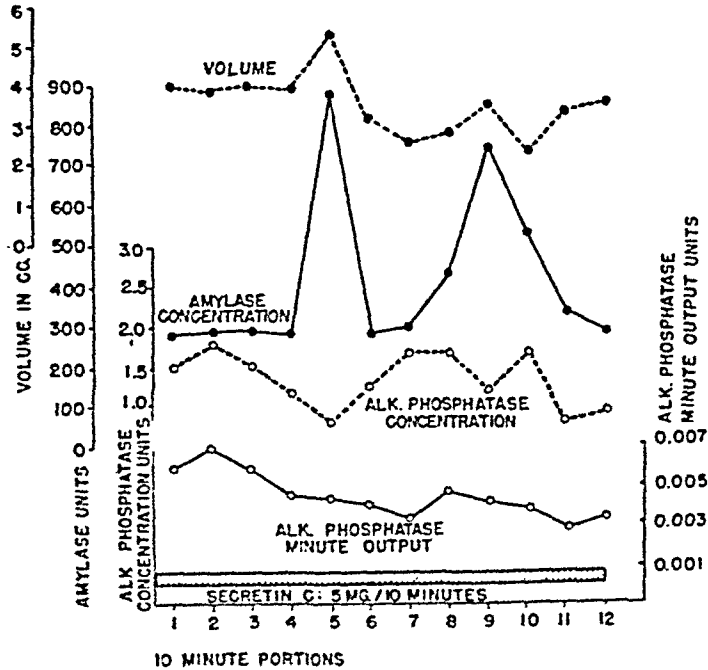


Fig. 5. Dog 6, 11 kg. Effect of pancreozymin on amylase and alkaline phosphatase. Twenty mg. of pancreozymin was injected after portion 4 and 14 mg. after portion 8.

DISCUSSION

It is well known that much of the earlier work on the action of secretin suffered from the use of relatively crude preparations containing either histamine-like sub-

stances or other vasodepressors which in themselves affect the pancreatic secretion. Of greater importance to this kind of study was the fact that many of these earlier secretin preparations contained varying amounts of pancreozymin. Barrington's (9) finding that his secretin preparations stimulated enzyme secretion can be explained by the fact that he used SI, a secretion preparation which is known to contain pancreozymin. Care was therefore taken to use in the present work highly purified secretin preparations in which the content of pancreozymin can be considered to be minimal or absent and which were shown to be free of vasodepressor action.

In the study of the effect of secretin on the enzyme production of the pancreas, the 'continuous' secretin injection provides an ideal method of approach because a continuous uniform secretion would serve to distinguish a 'washing out' process from a real production of enzymes. The results observed clearly indicate that the increase of secretin administration did not bring about a continuous increase in enzyme output. Here, the effect of histamine on the pepsin response of gastric secretion (22) is in contrast. The minute output of pepsin in response to large doses of histamine was at all times significantly larger (although the concentration of pepsin fell) than that in response to small doses and the effect of the higher dose of histamine, in increasing pepsin output, tended to continue after resumption of a lower dose of histamine. It seems to be conclusive from our results that secretin has no stimulating effect on amylase production by the pancreas.

These results suggest that there is a constant basal rate of secretion of amylase (and probably of the other pancreatic enzymes). When little or no water and bicarbonate are being secreted this amylase accumulates within the gland tubules, to be 'washed out' in the first portion of juice secreted after a period of quiescence. When secretin is the sole stimulus acting upon the pancreas, this basal rate of enzyme secretion continues unchanged and the concentration of amylase in the juice depends simply on the amount of water which the secretin causes the gland to secrete and thus to be available for dissolving the fixed amount of amylase.

We have previously shown that with the degeneration of the ductule cells that occurs in association with prolonged alloxan diabetes, a diminution in the volume of juice secreted in response to secretin occurs (24). This finding suggested that these ductule cells might be the site where secretin acts to stimulate secretion of water and bicarbonate. The present studies neither support nor disprove this possibility. They do show that at least one element of pancreatic juice, namely alkaline phosphatase, probably is secreted by the ductule cells.

It was shown in this work that both secretin and pancreozymin have no effect on alkaline phosphatase production in the pancreas. This observation suggests that the source of enzyme production is probably not the same for phosphatase as for the other three major enzymes. This supports Jacoby's proposal (16) that the ductule cells are responsible for the production of the phosphatase, whereas the acinar cells secrete the other three enzymes, namely the amylase, trypsinogen and lipase, since the alkaline phosphatase reaction is always positive in the ductule cells of the dog's pancreas, and negative in the glandular cells.

In the case of the secretion of alkaline phosphatase by the liver into the bile, there are two theories concerning the origin of the enzyme (23). One theory holds that the alkaline phosphatase of the blood serum is excreted into the bile; the other states



that the alkaline phosphatase which appears in the bile is formed by the liver cells. No decision can be made as to which of these mechanisms applies to the secretion of alkaline phosphatase by the pancreas.

#### SUMMARY

The effect of secretin and pancreozymin on amylase and alkaline phosphatase secretion by the dog's pancreas has been observed by using highly purified secretin and pancreozymin. A 'continuous' secretin injection method was used. The results indicate that an increase of secretin administration causes an increase in the volume of juice but does not bring about an increase in the output of the enzyme per minute. Secretin, therefore, has no stimulating effect on enzyme production of the pancreas, neither amylase nor alkaline phosphatase. Pancreozymin stimulates the amylase output of the pancreas, but not alkaline phosphatase.

The observations indicate that the source of enzyme secretion is probably not the same for phosphatase as for the other three major enzymes. The proposal suggested by Jacoby (16) that the ductule cells are responsible for the production of the phosphatase and the acinar cells for the other three enzymes has thus been supported.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOLUME 154

September 1, 1948

NUMBER 3

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## DERIVATION OF LEADS I AND III IN THE DOG FROM AN ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VR, VL, AND VF<sup>1</sup>

L. H. NAHUM, H. M. CHERNOFF<sup>2</sup> AND W. KAUFMAN

*From the Laboratory of Physiology, Yale University School of Medicine*

NEW HAVEN, CONNECTICUT

**B**EAM deflections in the standard limb leads I and III result when potential differences exist between the two extremity electrodes employed in recording each of these leads. By convention, upward movement of the beam in lead I occurs when the potential at the right forelimb is negative relative to that at the left forelimb, while movement of the beam downward occurs when the potential at the right forelimb is positive relative to that at the left. Similarly, in lead III upward movement of the beam occurs when the left forelimb is negative relative to the left hindlimb, while downward movement occurs when the potential at the left forelimb is positive relative to that at the left hindlimb. As Goldberger has pointed out, standard limb leads record only differences in potential between two given extremities and, therefore, on theoretical grounds, an upward movement of the beam will result when any one of five different combinations of potentials exists at a given moment at these extremities (1). Similarly, downward movement of the beam will occur when any one of five different combinations of potentials exists at the two extremities. When the beam remains at the isoelectric line in a standard limb lead during the depolarization process in a portion of the ventricle, the potentials developed at each of the two extremities must be of the same electrical sign and of equal magnitude. This will occur when any one of the following three possibilities exists: *a*) negative potentials of equal magnitude at both extremities; *b*) positive potentials of equal magnitude at both extremities; *c*) zero potential at both extremities.

Received for publication June 28, 1948.

<sup>1</sup> Aided by grants from the Fluid Research Fund, Yale University School of Medicine and from the U. S. Public Health Service.

<sup>2</sup> Senior Research Fellow, U. S. Public Health Service.

Hoff, Nahum and Kaufman (2-5) have previously demonstrated that upward movements of the beam in lead I in the dog resulted mainly from depolarization of the posterior right ventricle, while downward movements resulted from depolarization of the anterior left ventricle. Depolarization of the remaining regions of the ventricles was found not to cause deflections of the beam in lead I. Similarly, upward movements of the beam in lead III were attributed to depolarization of the anterior right ventricle, while downward movements took place when the posterior left ventricle was depolarized. Depolarization occurring in the rest of the ventricles produced minimal or no effect upon beam movement in lead III. The entire ventricular complex in both leads I and III was considered by these investigators to result from the interaction of oppositely directed electrical forces derived from the depolarization of specific regions of the right and left ventricles, and that these regions included the entire thickness of the myocardium in either ventricle (6-8). This explanation of the genesis of the QRS complex in leads I and III, based upon experimental observations, differs from the conclusions reached by Gardberg and Ashman (9) who attempted to visualize the ventricular complex in terms of the advance and retreat of anatomically localized dipoles. They concluded that potentials resulting from excitation of the right ventricle are not recognizable as such in the electrocardiogram, except perhaps at the very onset or at the termination of ventricular excitation when the unopposed vectors resulting from the spread of the excitatory process initially through the septum from left to right and terminally in the pulmonary conus dominate and are thus responsible for the inscription of a Q-wave and S-wave, respectively. According to their analysis, it is the spread of excitation through the left ventricle from endocardium to epicardium which primarily accounts for both upstroke and downstroke in the ventricular complex in leads I and III. The experiments to be described below offer no support for the theoretical explanation given by Gardberg and Ashman for the genesis of the QRS complex in the standard limb leads, and confirm and extend the views first expressed by Hoff, Nahum and Kaufman.

Since any given beam movement recorded in the standard limb leads may result from any one of five different combinations of potentials existing at the two extremities employed in recording the lead, a more exact analysis of the derivation of leads I and III requires knowledge of the origin and nature of the instantaneous potentials developed independently at each limb throughout the cardiac cycle. This knowledge is now available from recent experimental studies in the dog on the nature of the 'unipolar' extremity leads VR, VL and VF (10-12). In those studies it was found that the ventricular complex in each 'unipolar' extremity lead represented the algebraic summation of oppositely directed potentials derived from the depolarization of specific proximal and distal ventricular zones. The spatial distribution of these ventricular zones was found to differ for each 'unipolar' extremity lead. Figure 1 shows a schematic representation of the heart surface as it was experimentally found to be divided into proximal, intermediate, and distal zones for each of the three 'unipolar' extremity leads. It can be seen that any segment of the heart which lies in the proximal zone of one extremity lead is also situated in the proximal, intermediate or distal zones of the other two extremity leads. It is possible to localize the site of preponderant depolarization during any given time interval to the proximal, intermediate or distal zone of a given 'unipolar' extremity lead from the direction of beam movement in that lead during that interval. Downward movement of the beam localizes the process to the proximal zone of the lead while upward movement of the beam localizes it to the distal zone. An isoelectric beam indicates either depolarization in the intermediate zone of the lead or equal degree of depolarization in both proximal and distal zones.

In any given 'unipolar' extremity lead, however, the proximal and distal zones are each relatively large and more specific localization within a given zone becomes possible only from study of the direction of beam movement in each of several 'unipolar' leads recorded simultaneously. For example, if during the same time interval the beam is moving downward in each of two leads, the depolarization process is more specifically localized to that region of the heart which lies in the proximal zone of both leads (all segments labelled *PP* in fig. 1). On the other hand, if the beam moves downward in one lead, but upward in the second lead, the site of preponderant depolarization is localized to that region of the heart which lies in the proximal zone of the first lead, but in the distal zone of the second (all segments labelled *PD* in fig. 1). By study of the three 'unipolar' extremity leads, VR, VL, and VF, recorded simultaneously, even more discrete localization becomes possible. For example, if during the same time interval the electrocardiographic beam is moving downward

in both VR and VL, but upward in VF, the depolarization process is localized to that segment of the heart which is labelled *PPD* in figure 1. Conversely, if the beam is moving upward in both VR and VL, but downward in VF, the process is localized to that segment of the heart labelled *DDP* in figure 1. By similar analysis, any combination of beam movements in VR, VL, and VF during any small time interval will localize the site of preponderant depolarization during that interval to one or another of the various segments depicted in figure 1.

It should be remembered that each 'unipolar' extremity lead records the instantaneous differences in potential which exist throughout the cardiac cycle between the exploring electrode and the central terminal electrode. Leads taken in such a manner may not be truly unipolar if the central terminal electrode is not at zero potential at all times during the cardiac cycle. However, if the potential of the central terminal electrode, whatever it may be, is considered at any moment during the cardiac cycle to be the same in each lead when these leads are recorded simultaneously, lead I may be derived from the equation  $\text{lead I} = \text{lead VL} - \text{lead VR}$ , and lead III may be derived from the equation  $\text{lead III} = \text{lead VF} - \text{lead VL}$ .

By definition:

- 1) Lead I = potential at left arm (LA) — potential at right arm (RA)
- 2) Lead III = " " left leg (LL) — " " left arm (LA)
- 3) Lead VR = " " right arm (RA) — " " central terminal
- 4) Lead VL = " " left arm (LA) — " " " "
- 5) Lead VF = " " left leg (LL) — " " " "

Equations 1 and 2 may therefore be rewritten as follows:

$$\begin{aligned}\text{Lead I} &= (\text{Lead VL} + \text{potential C.T.}) - (\text{Lead VR} + \text{potential C.T.}) \\ \text{Lead III} &= (\text{Lead VF} + \text{potential C.T.}) - (\text{Lead VL} + \text{potential C.T.})\end{aligned}$$

If the contribution of the central terminal to the potential differences recorded in each of the three 'unipolar' extremity leads is considered to be the same at any given moment when these leads are recorded simultaneously, it follows that  $\text{Lead I} = \text{Lead VL} - \text{Lead VR}$  and that  $\text{Lead III} = \text{Lead VF} - \text{Lead VL}$ .

Thus, knowing the nature of leads VR, VL, and VF, these equations should permit one to learn the reasons why normal and/or injury potentials from certain specific regions of the heart are maximally recorded in one or another of the standard limb leads I and III, while similar potentials from other specific regions are minimally or not at all recorded in one or another of these leads. Lead II is not analyzed in this study because by definition  $\text{lead II} = \text{lead I} + \text{lead III}$ .

The experimental methods employed in the following studies have been described in detail in previous communications concerning the nature of "unipolar" extremity leads (10, 11, 12).

#### DERIVATION OF LEAD I FROM ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VR AND VL

##### *A. Regions of the Heart in Which Depolarization Results in Maximal Deflections of the Beam in Lead I*

Exploration of the ventricular surfaces of the heart by various techniques reveals that the greatest upward deflections of the beam in lead I occur when the mid-third of the posterior right ventricle is depolarized (segment 1, fig. 2A), while the greatest downward deflections in this lead result from depolarization of the mid-third of the anterior left ventricle (segment 6, fig. 2A). The explanation for these observed findings becomes apparent from a study of the changes that occur in leads VR and VL during the depolarization and repolarization of these heart segments.



development during diastole of potentials at the right forelimb which are positive relative to those developed at the left forelimb. In lead I, therefore, downward displacement of the diastolic baseline occurs and an ST-segment elevation is recorded (fig. 5A).

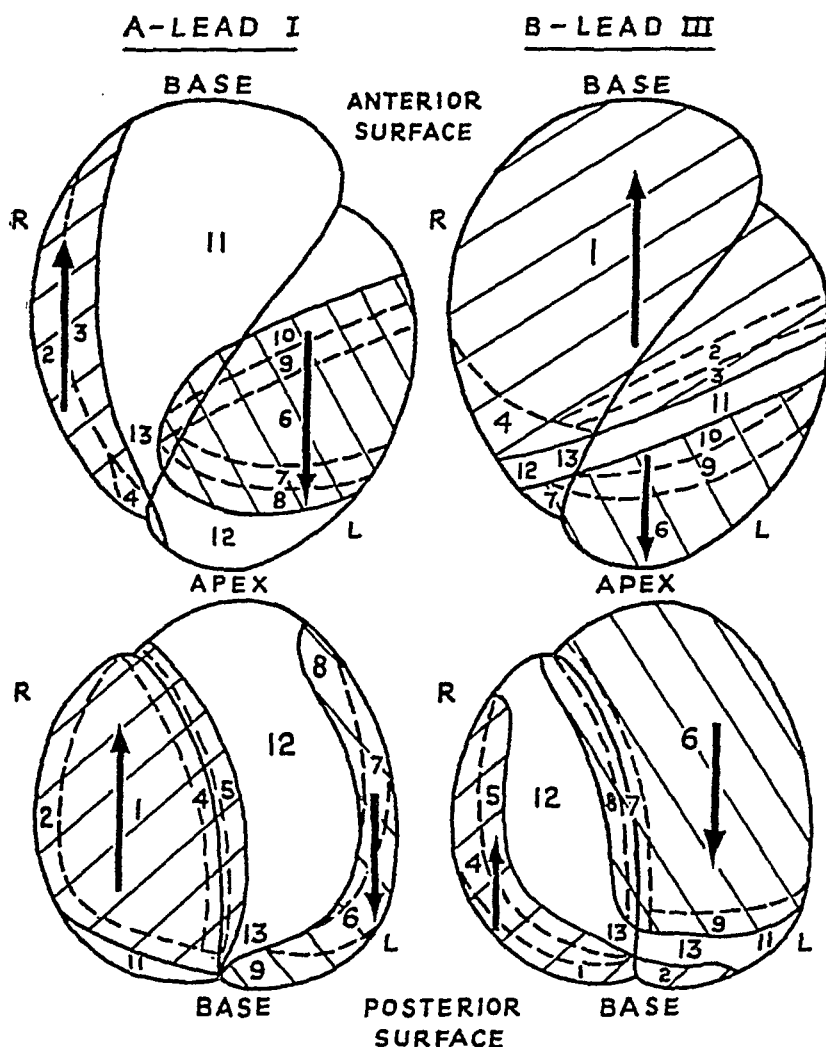


Fig. 2. DRAWING OF ANTERIOR AND POSTERIOR HEART SURFACE in the dog showing the electrically active and neutral regions for leads I and III. The lined areas represent the electrically active regions for leads I and III. The arrows point in the direction of beam movement in these leads when these regions are depolarized. The blank areas represent the electrically neutral regions for leads I and III. A. The numbers identify the specific segments whose depolarization produces the thirteen different combinations of potentials at the right and left forelimbs, as described in the text. B. The numbers identify the specific segments whose depolarization produces the 13 different combinations of potentials at the left forelimb and left hindlimb, as described in the text.

3. Acceleration of repolarization of the mid-third of the posterior right ventricle by heating results in increased positivity of the T-wave in VR, but in increased negativity of T-VL. The increase in the height of T-VR is due to increase in the magnitude of the positive potential developed at the right forelimb, while the deep inversion of the T-VL is due to increase in the magnitude of negative potential developed at the left forelimb. Inasmuch as the right forelimb is initially positive

with respect to the left, the beam in lead I is initially deflected downward, inscribing an inverted T-wave (fig. 6). Decelerating the repolarization of this region by cooling results in increased negativity of T-VR and in increased positivity of T-VL. Because the right forelimb becomes initially negative relative to the left, the beam in lead I

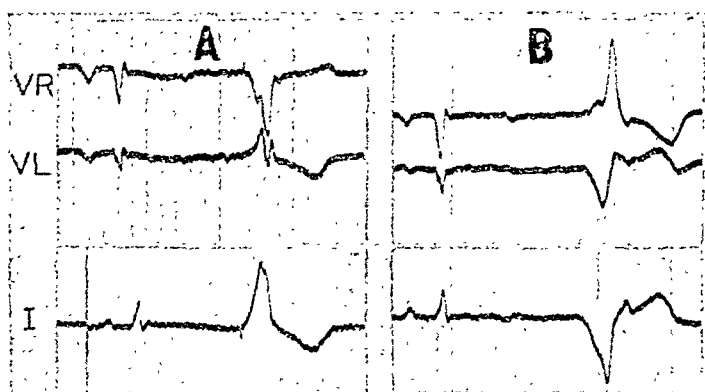


Fig. 3. 7.0 K. MALE DOG, April 8, 1948. *A. Forced extrasystoles from right posterior ventricle* The fine vertical lines represent 0.04-sec. time intervals. Following the stimulus artefact, the initial movement of the beam is downward in VR, upward in VL, and upward in lead I. *B. Forced extrasystoles from left anterior ventricle.* The initial movement of the beam is upward in VR, downward in VL and downward in lead I.

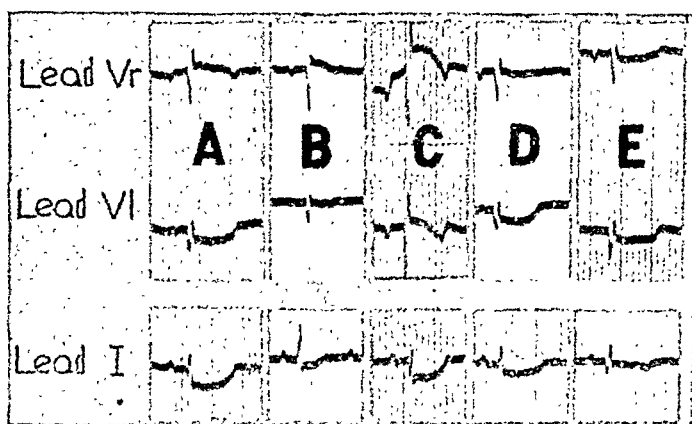


Fig. 4. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VR and VL that produce ST-segment depression in lead I. *A.* 5.6 K. female dog, Aug. 21, 1947. M/5 KCl applied to segment 1, fig. 2A. The ST-segment is elevated in VR, depressed in VL and sharply depressed in lead I. *B.* 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment 2, fig. 2A. The ST-segment is elevated in VR, isoelectric in VL and moderately depressed in lead I. *C.* 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 3, fig. 2A. The ST-segment is elevated in both VR and VL, but to a greater extent in VR. ST-segment in lead I moderately depressed. *D.* 5.6 K. female dog, Aug. 21, 1947. M/5 KCl applied to segment 4, fig. 2A. ST-segment isoelectric in VR, depressed in VL and depressed in lead I. *E.* 8.7 K. male dog, Aug. 7, 1947. M/5 KCl applied to segment 5, fig. 2A. ST-segment depressed in both VR and VL, but to a greater extent in VL. Minimal depression of ST-I. Control records are not exhibited here, but all leads showed isoelectric ST-segments.

is initially deflected upward, inscribing an upright T-I (fig. 6). Alterations of the speed of repolarization by similar treatment of the mid-third of the anterior left ventricle results in opposite changes in T-VR, T-VL, and T-I (fig. 6).

The above experimental findings all lead to the same conclusion, namely, that depolarization of the mid-third of the posterior right ventricle results in the development of potentials at the right forelimb which are negative with respect to those

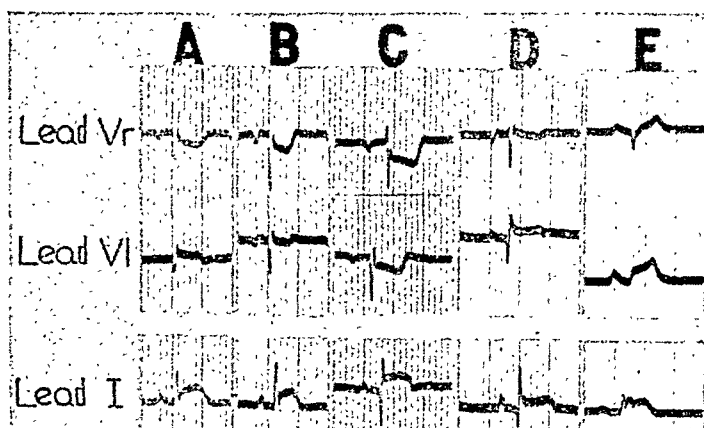
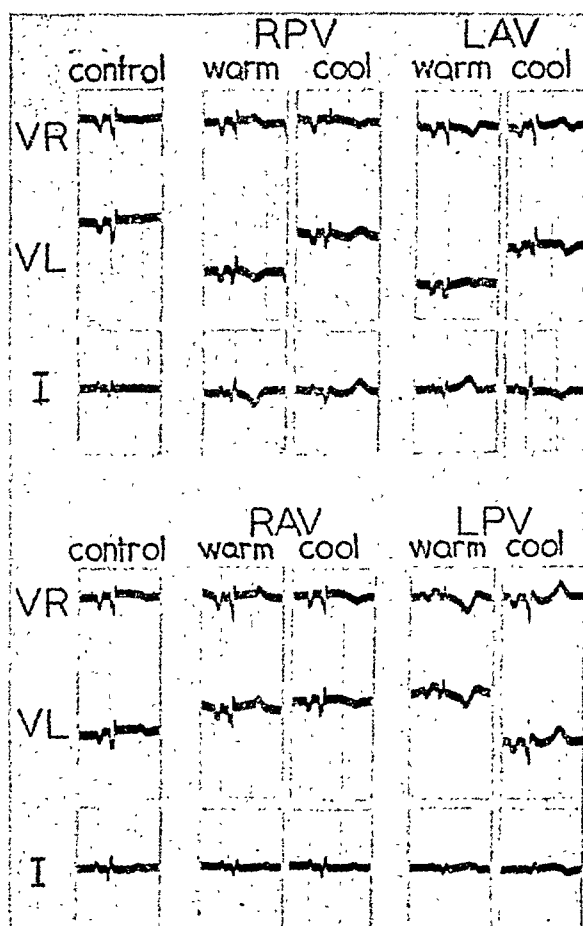


Fig. 5. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VR and VL that produce ST-segment elevation in lead I. A. 7.2 K. female dog, Sept. 21, 1947. M/5 KCl applied to segment 6, fig. 2A. The ST-segment is depressed in VR, elevated in VL and elevated in lead I. B. 6.7 K. male dog, Sept. 11, 1947. M/5 KCl applied to segment 7, fig. 2A. The ST-segment is depressed in VR, isoelectric in VL and elevated in lead I. C. 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 8, fig. 2A. The ST-segment is depressed in both VR and VL, but to a greater extent in VR. ST-I is elevated. D. 8.0 K. male dog, Sept. 26, 1947. M/5 KCl applied to segment 9, fig. 2A. The ST-segment is isoelectric in VR, moderately elevated in VL, and moderately elevated in lead I. E. 8.7 K. male dog, Aug. 7, 1947. M/5 KCl applied to segment 10, fig. 2A. The ST-segment is elevated in both VR and VL, but to a greater extent in VL. ST-I is moderately elevated. Control records are not exhibited, but all showed isoelectric ST-segments.

Fig. 6. CHANGES IN T-WAVE in leads VR, VL, and I following heating and cooling the heart surface. 7.2 kg. male dog, May 18, 1948. RPV = right posterior ventricle; LAV = left anterior ventricle; RAV = right anterior ventricle; LPV = Left posterior ventricle. Warming RPV makes T-VR more positive, T-VL more negative, and T-I sharply inverted. Cooling RPV makes T-VR more negative, T-VL more positive, and T-I sharply upright. Warming LAV makes T-VR more negative, T-VL more positive, and T-I sharply upright. Cooling LAV makes T-VR more positive, T-VL more negative, and T-I sharply inverted. Warming RAV makes both T-VR and T-VL more positive. T-I shows no change. Cooling RAV makes both T-VR and T-VL more negative. T-I shows no change. Cooling RAV makes both T-VR and T-VL more negative. T-I shows no change. Warming LPV makes both T-VR and T-VL more negative. T-I shows little change. Cooling LPV makes both T-VR and T-VL more positive. T-I shows only slight change.



developed at the left forelimb, and that depolarization of the mid-third of the anterior left ventricle results in the development of potentials at the right forelimb which are



positive in relation to those developed at the left forelimb. Since in each case the potentials at the two extremities are of opposite electrical sign, lead I, recording the difference in potential between the two extremities, exhibits maximal deflections of the beam upward and downward respectively following the depolarization of these two heart segments.

*B. Regions of the Heart in Which Depolarization Results in Deflections of the Beam in Lead I of Lesser Magnitude Than Those Described in Section A*

Upward deflections of the beam in lead I have also been found to result when those regions of the heart which border immediately upon the mid-third of the posterior right ventricle become depolarized, while downward deflections also result from depolarization of those regions that border upon the mid-third of the anterior left ventricle, but these deflections are of lesser magnitude than those which result from depolarization of the mid-third of the posterior right and anterior left ventricles. Study of the nature of the ST-segment displacements in leads VR, VL, and I resulting from experimental injury to these specific regions furnishes the explanation for these observations.

1. M/5 KCl solution applied to the epicardial surface of heart segment 2, figure 2A, produces ST-segment elevation in VR but no displacement of ST-VL, indicating that during diastole the right forelimb is negative relative to the left. Lead I reflects this relationship by an upward displacement of the diastolic baseline and hence exhibits a depressed ST-segment (fig. 4B).

2. M/5 KCl solution applied to heart segment 3, figure 2A, produces ST-segment elevation in both VR and VL, but the degree of upward displacement of the ST-segment is greater in VR than in VL, indicating that the degree of negativity at the right forelimb during diastole is greater than that at the left forelimb. During diastole the right forelimb is negative relative to the left, and therefore lead I exhibits upward displacement of the diastolic baseline (ST-segment depression, fig. 4C).

3. M/5 KCl solution applied to segment 4, figure 2A, produces no displacement of ST-VR, but depression of ST-VL, indicating that during diastole the right forelimb is negative relative to the left. Lead I, therefore, exhibits ST-segment depression (fig. 4D).

4. M/5 KCl solution applied to segment 5, figure 2A, produces ST-segment depression in both VR and VL, but the degree of depression is somewhat greater in VL than in VR. Although both extremities are at positive potential during diastole, the right forelimb is somewhat negative relative to the left, and lead I, therefore, exhibits very slight depression of the ST-segment (fig. 4E).

5. M/5 KCl solution applied to segment 7, figure 2A, produces depression of ST-VR but no displacement of ST-VL, indicating that during diastole the right forelimb is positive relative to the left. In lead I, therefore, there is downward displacement of the diastolic baseline (ST-segment elevation, fig. 5B).

6. M/5 KCl solution applied to segment 8, figure 2A, produces depression of the ST-segment in both VR and VL, but the degree of downward displacement is greater in VR than in VL. The right forelimb is thus relatively positive with respect to the left during diastole, and lead I therefore exhibits downward displacement of the diastolic baseline (ST-segment elevation, fig. 5C).

7. M/5 KCl solution applied to segment 9, figure 2A, produces no displacement of ST-VR, but produces moderate elevation of ST-VL. The right forelimb is thus positive relative to the left during diastole, and therefore lead I exhibits slight ST-segment elevation (fig. 5D).

8. M/5 KCl solution applied to segment 10, figure 2A, produces ST-segment elevation in both VR and VL, but the degree of upward displacement is greater in VL than in VR. The right forelimb is thus somewhat positive relative to the left during diastole, and lead I therefore exhibits moderate ST-segment elevation (fig. 5E).

The five possible combinations of positive, zero, and negative potentials at the right and left forelimbs that could account theoretically for an upward movement of the beam in lead I have all been found to exist and each combination has been demonstrated to result from the depolarization of specific portions of the posterior right ventricle, posterior right apex, right lateral wall, and the posterior septum (segments 1-5, fig. 2A). The greatest difference in potential between the two extremities as recorded in lead I has been shown to develop following depolarization of the mid-third of the posterior right ventricle (segment 1, fig. 2A) while differences of lesser magnitude, but of the same electrical sign, result from the depolarization of the immediately adjacent regions. Similarly, the five possible combinations of potential at the right and left forelimbs that could account theoretically for a downward movement of the beam in lead I have each been demonstrated to result from the depolarization of specific portions of the anterior left ventricle, the mid-third of the anterior septum, a small portion of the lower third of the anterior right ventricle near the septum, the upper two-thirds of the lateral wall of the left ventricle, and a small segment of the basal posterior left ventricle (segments 6-10, fig. 2A). The mid-third of the anterior left ventricle (segment 6, fig. 2A) was found to be the region whose depolarization results in the development of the greatest difference in potential between the right and left forelimbs, while depolarization of the immediately surrounding regions results in differences of lesser magnitude, but of similar electrical sign.

More accurate localization of injuries to the dog heart is possible from study of the ST-segment displacements in leads VR and VL than from a study of ST-segment displacement in lead I alone. ST-segment elevation in lead I localizes the injury only to the general region of the anterior left ventricle, while the direction of ST-segment displacements in VR and VL helps localize the injury to more specific parts of this general region. ST-segment depression in lead I localizes the injury only to the general region of the posterior right ventricle, while displacements of ST-VR and ST-VL help localize the injury to more specific parts of this region.

### *C. Regions of the Heart in Which Depolarization Results in Minimal or no Deflections of the Beam in Lead I*

Exploration of the ventricular surfaces of the heart reveals that there exist specific regions in which depolarization fails to produce any appreciable deflection of the beam in lead I. These segments are the mid and upper thirds of the anterior right ventricle, the upper third of the anterior septum, the basal portion of the anterior left ventricle, the left apex, and the lower two thirds of the posterior left ventricle (segments 11-13, fig. 2A). As has been mentioned above, there are three

possible conditions in which an isoelectric beam can occur in a standard limb lead even though certain segments of the heart are the site of depolarization at the moment. In each instance there is no appreciable potential difference developed between the two extremities as a result of such depolarization. The reasons for the relative or absolute lack of electrical representation in lead I of the heart segments listed above may be determined from a study of the changes occurring in leads VR and VL following excitation, injury, or alteration in rate of repolarization of these segments.

1. *Mid and upper thirds of anterior right ventricle (segment 11, fig. 2A).* a) M/5 KCl solution applied to this region produces ST-segment elevation of the same degree in both VR and VL, indicating that the potentials which develop at each extremity during diastole as a result of the current of injury are each negative and of the same magnitude. Because there is no difference in potential between the two limbs during diastole, the ST-segment in lead I remains isoelectric (fig. 7A).

b) Acceleration of the rate of repolarization of this segment by heat makes the T-wave become more upright in both VR and VL. Because the degree of

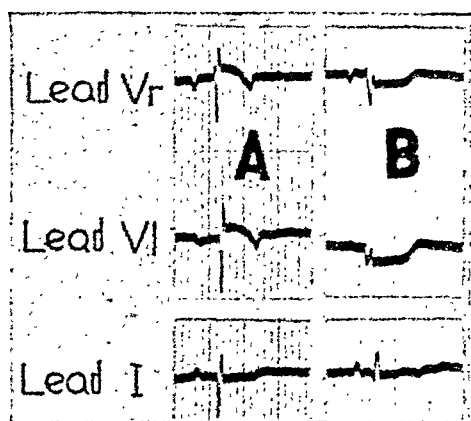


Fig. 7. ST-SEGMENT DISPLACEMENTS in VR and VL that fail to produce ST-segment change in lead I. A. 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 11, fig. 2A. The ST-segment is elevated to the same extent in both VR and VL. The ST-segment in lead I remains isoelectric. B. 5.6 K. male dog, Aug. 21, 1947. M/5 KCl applied to segment 12, fig. 2A. The ST-segment is depressed to the same extent in both VR and VL. The ST-segment in lead I remains isoelectric. Control records are not exhibited, but all leads showed isoelectric ST-segments.

change in the amplitude of the T-wave in each of these leads tends to be of equal magnitude, the difference in potential between the two forelimbs throughout the repolarization process after treatment with heat remains the same as that which existed before the heating. The amplitude and direction of the T-wave in lead I therefore tends to remain unchanged despite the fact that the rate of repolarization of a large portion of the heart has been materially altered (fig. 6). Conversely, cooling this region results in inversion of the T-waves in both VR and VL, but because the degree of change is the same in each of these leads, the T-wave in lead I tends to remain unaltered (fig. 6).

2. *Left apex and lower two-thirds of posterior left ventricle (segment 12, fig. 2A).* a) M/5 KCl solution applied to this region produces ST-segment depression of the same degree in both VR and VL, indicating that positive potentials of equal magnitude are developed at each forelimb during diastole as a result of the injury current. No difference in potential has developed between the two extremities during diastole, and therefore lead I reveals an isoelectric ST-segment (fig. 7B).

b) When extrasystoles were elicited by stimulation of the epicardial surface of the lower third of the posterior left ventricle, the beam in lead I remained at the iso-

electric line for about 0.04 sec. before moving downward (fig. 8). During this 0.04-sec. interval, however, the beam in lead III rapidly moved downward. The explanation for this isopotential period in lead I (at a time when some parts of the heart were undergoing activation) becomes clear from an analysis of the configuration of the extrasystole as it was simultaneously inscribed in VR and VL. It can be seen in both VR and VL that the beam initially moved upwards with identical slopes, indicating that the potentials developed at each forelimb at each instant during this 0.04-sec. period were of the same electrical sign and of equal magnitude. Because no difference in potential developed between the two extremities as a result of the early activation of the posterior left ventricle, the beam in lead I, therefore, remained at the isoelectric line until the excitatory process had spread to other parts of the heart.

c) When repolarization of this heart segment is accelerated by heat, the T-wave in both VR and VL becomes more negative, the degree of change in the T-wave in

Fig. 8. FORCED EXTRASYSTOLE from lower third of posterior left ventricle. 9.0 K. male dog, Oct. 14, 1947. The initial movement of the beam is upward in both VR and VL and the slope of the upward deflection is the same in each lead during the 0.04-sec. period following the stimulus artefact. The initial movement of the beam is downward in lead VF and immediately follows the stimulus artefact. The beam remains at the isoelectric line in lead I until 0.04 sec. after the stimulus artefact. In lead III the beam is deflected downward immediately after the stimulus artefact.



each of these leads being equal (fig. 6). The T-wave in lead I exhibits little or no alteration from the control value since the difference in potential between the two extremities throughout the repolarization process remains at the pretreatment level. Conversely, when repolarization of this region is delayed by cooling, both T-VR and T-VL become more upright. Because the degree of change in potential at both extremities tends to be of similar magnitude, the T-wave in lead I exhibits little or no change (fig. 6).

3. *Basal posterior left ventricle near the septum (segment 13, fig. 2A).* M/5 KCl solution applied to this segment produces no significant displacement of the ST-segment in either VR or VL, and therefore lead I exhibits an isoelectric ST-segment (fig. 9A, B). The reason for the lack of any displacement of the ST-segment in either VR or VL is that the injured region lies in the intermediate zone of each of these leads and is, therefore, in an electrically neutral area of both of these leads (fig. 1). In lead III, however, the ST-segment is displaced upward because the treated area lies within the proximal zone of lead VF (fig. 9A, B).

The above observations all demonstrate that potentials resulting from the

depolarization or repolarization of the major portion of the anterior right ventricle, the left apex, and the major portion of the posterior left ventricle fail to produce significant beam deflections in lead I.

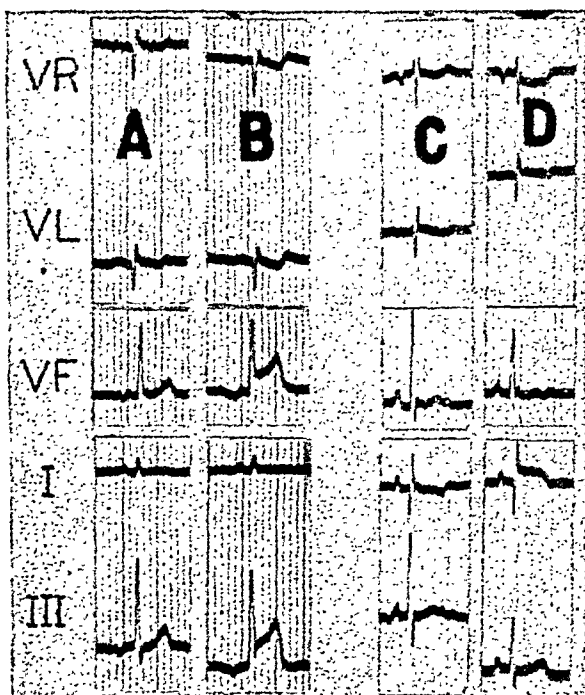


Fig. 9. REGIONS OF THE HEART in which injury produces ST-segment displacement in only one of the three unipolar leads. *A.* Control; 11.4 male dog, Oct. 14, 1947. *B.* M/5 KCl applied to segment 13, fig. 2A. The ST-segment tends to remain at the isoelectric line in VR and VL and lead I. The ST-segment is elevated in VF and lead III. *C.* Control; 6.5 K. male dog, Oct. 23, 1947. *D.* M/5 KCl applied to segments 1-3, 6, 9-11, fig. 2B. The ST-segment is depressed in VR, and isoelectric in VL and VF. Lead I exhibits ST-segment elevation. Lead III exhibits an isoelectric ST-segment.

#### SUMMARY AND CONCLUSIONS

The present study of the nature of lead I by analysis of the 'unipolar' extremity leads VR and VL permits the following conclusions:

1. The five possible combinations of potentials at the right and left forelimbs that could account theoretically for upward movement of the beam in lead I and the five possible combinations of potentials at these extremities that could account theoretically for downward movement of the beam in lead I have been shown to exist experimentally and each combination can be related to the depolarization of a specific ventricular region.
2. It is only when the site of preponderant depolarization lies within the anterior left ventricle that downward deflection of the beam occurs in the ventricular complex in lead I because only depolarization in this region results in the development of potentials at the right forelimb which are positive relative to those developed at the left forelimb. If a portion of this region is activated in advance of the rest of the ventricles, the initial movement of the beam in the ventricular complex will be downward and a Q-wave will, therefore, be inscribed in lead I. Later excitation of this region will also result in downward movement of the beam in lead I and this accounts for the downstroke of the R- and S-waves in this lead.
3. It is only when the site of preponderant depolarization lies within the posterior right ventricle and posterior septum that upward deflections of the beam occur in the ventricular complex in lead I because only depolarization of these regions gives rise to potentials at the right forelimb which are negative relative to those developed at the left forelimb. If a portion of these regions is activated early, the beam will move

upward and inscribe the upstroke of the Q- and R-waves in lead I. If a part of these regions is the last to be activated, the beam will again be deflected upward in lead I, thus inscribing the upstroke of the S-wave in this lead.

4. No deflections of the beam take place in lead I when the major portions of the anterior right ventricle, the left apex, and the posterior left ventricle become depolarized or repolarized, since during these events no appreciable difference in potential develops between the right and left forelimbs. Depolarization of the major portion of the anterior right ventricle results in the development of negative potentials of equal or approximately equal magnitude at both extremities, whereas depolarization of the major portion of the posterior left ventricle and left apex results in the development of positive potentials of equal or approximately equal magnitude at both extremities. There exists a small segment of the basal posterior left ventricle near the septum in which depolarization fails to produce beam movements in lead I because it fails to produce any deflections of the beam in either VR or VL.

5. An isopotential ST-segment in lead I can occur when any of the following conditions exists: *a*) no current of injury is present; *b*) injury is present in the mid-third of the anterior right ventricle and/or the lower two-thirds of the posterior left ventricle and left apex; *c*) repolarization has not yet begun in the anterior left and posterior right ventricles; *d*) the anterior left and posterior right ventricles are repolarizing at the same rate, thus producing potentials at each extremity of the same magnitude but of opposite electrical sign so that the net potential at each extremity is zero.

6. More specific localization of injury to the heart is possible from study of ST-segment displacements in VR and VL than from study of ST-segment displacement in lead I alone. ST-segment elevation in lead I localizes the injury only to the general region of the anterior left ventricle, while the displacements of the ST-segments in VR and VL help localize the injury to more specific parts of this region. ST-segment depression in lead I localizes injury only to the general region of the posterior right ventricle, while the displacements of the ST-segments in VR and VL help localize the injury to more specific parts of this region.

7. When the anterior left ventricle repolarizes in advance of the posterior right ventricle, the T-wave is upright in lead I because of the development of initial relative negativity at the right forelimb. Conversely, when the posterior right ventricle repolarizes in advance of the anterior left ventricle, the T-wave in lead I is inverted because of the development of initial relative positivity at the right forelimb. The repolarization of the major portions of the anterior right and posterior left ventricles contributes little or nothing to the configuration of the T-wave in lead I because the repolarization of each of these regions tends to produce simultaneous potentials of the same magnitude and electrical sign at the right and left forelimbs.

These conclusions as to the derivation of lead I in the dog correspond closely with the findings of Hoff, Nahum and Kaufman (2-5) on the nature of the various components of the ventricular complex in this lead. These authors described lead I as the algebraic summation of potentials derived mainly from the depolarization of the posterior right and anterior left ventricles. They found that the depolarization of the anterior right and posterior left ventricles was not represented in lead I. The

present study has more exactly delimited the electrically active as well as the electrically neutral areas for lead I, and has established the basis for the presence or absence of electrical representation of the various regions of the heart in lead I.

#### DERIVATION OF LEAD III FROM ANALYSIS OF SIMULTANEOUSLY RECORDED LEADS VF AND VL

##### *A. Regions of the Heart in Which Depolarization Results in Maximal Deflections of the Beam in Lead III*

Exploration of the ventricular surfaces of the dog heart reveals that the greatest upward deflections of the beam in lead III occur when the upper half of the anterior right ventricle (segment 1, fig. 2B) becomes depolarized while the greatest downward deflections in this lead result when the left apex and lower two thirds of the posterior

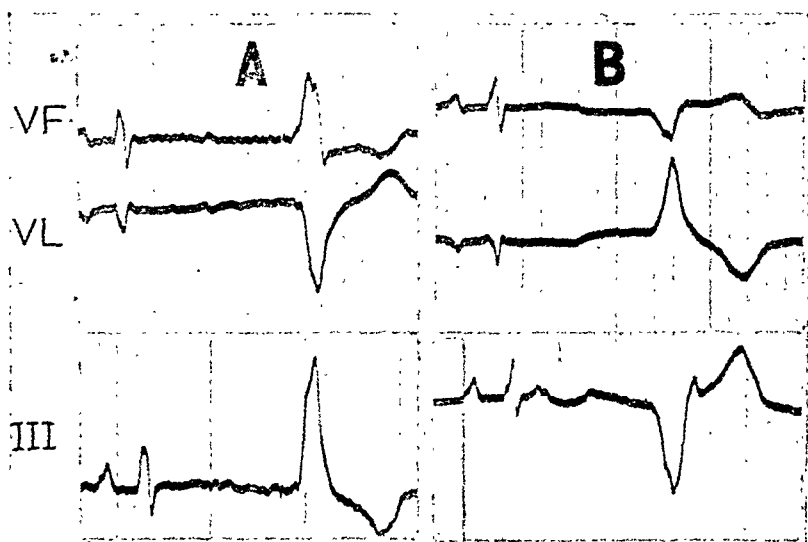


Fig. 10. 7.0 K. MALE DOG, Apr. 8, 1948. *A. Forced extrasystoles from anterior right ventricle.* The fine vertical lines represent 0.04-sec. time intervals. Following the stimulus artefact, the initial movement of the beam is upward in lead VF, downward in lead VL and upward in lead III. *B. Forced extrasystoles from posterior left ventricle.* Following the stimulus artefact the initial movement of the beam is downward in lead VF, upward in lead VL and downward in lead III.

left ventricle are depolarized (segment 6, fig. 2B). The explanation for these observations becomes apparent from a study of the changes that occur in the 'unipolar' extremity leads VF and VL following the depolarization and repolarization of these segments.

1. Depolarization initiated in the anterior right ventricle by epicardial stimulation results in oppositely directed initial deflections of the beam in VF and VL, indicating that the potentials developed at each extremity are initially of opposite electrical sign, the left forelimb being initially negative with respect to the left hindlimb. Lead III, therefore, exhibits an initial upward deflection (fig. 10A). Conversely, stimulation of the left apex and lower two thirds of the posterior left ventricle results in initial downward deflection of the beam in VF but in initial upward movement of the beam in VL, indicating that the left forelimb is initially positive with respect to the left hindlimb. Lead III, therefore, exhibits an initial downward deflection (fig. 10B).

2. M/5 KCl solution applied to the surface of the anterior right ventricle (segment 1, fig. 2B) produces ST-segment elevation in VL and ST-segment depression in VF, indicating that injury to this region results in the development during diastole of potentials at the left forelimb which are negative relative to those developed at the left hindlimb. Lead III, therefore, exhibits an upward displacement of the diastolic baseline or ST-segment depression (fig. 11A). When M/5 KCl solution is applied to the surface of the left apex or lower two-thirds of the posterior left ventricle (segment 6, fig. 2B), ST-segment depression occurs in VL, while ST-segment elevation occurs in VF. This indicates that injury to this region of the heart results in the development during diastole of potentials at the left forelimb which are

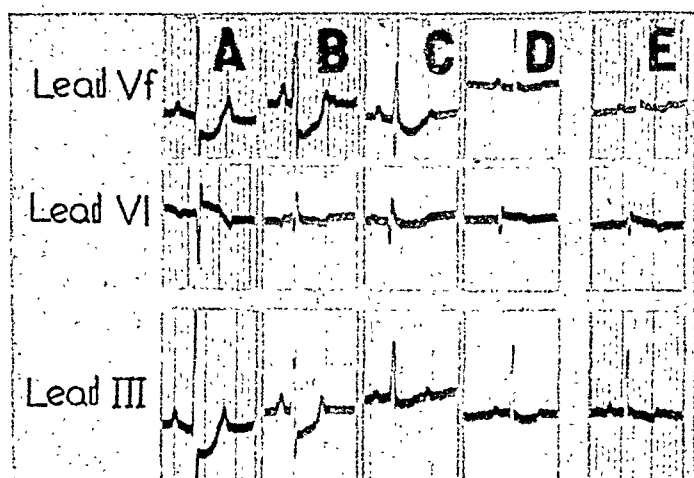


Fig. 11. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VF and VL that produce ST-segment depression in lead III. A. 9.6 K. male dog, July 31, 1947. M/5 KCl applied to segment 1, fig. 2B. The ST-segment is depressed in VF, elevated in VL and depressed in lead III. B. 7.5 male dog, Jan. 6, 1948. M/5 KCl applied to segment 4, fig. 2B. The ST-segment is depressed in VF, isoelectric in VL and depressed in lead III. C. 8.0 K. male dog, Sept. 26, 1947. M/5 KCl applied to segment 5, fig. 2B. The ST-segment is depressed both in VF and VL, but to a greater extent in VF. ST-III is moderately depressed. D. 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment, 2, fig. 2B. The ST-segment is isoelectric in VF, moderately elevated in VL and moderately depressed in lead III. E. 6.6 K. male dog, Sept. 2, 1947. M/5 KCl applied to segment 3, fig. 2B. The ST-segment is elevated both in VF and VL, but to a slightly greater extent in VL. ST-III shows minimal depression. Control records are not exhibited but all leads showed isoelectric ST-segments.

positive with respect to those developed at the left hindlimb. In lead III, therefore, the diastolic baseline is displaced downward and ST-segment elevation results (fig. 12A).

3. Acceleration of repolarization of the upper half of the anterior right ventricle by heat results in increased positivity of the T-wave in VL, but in increased negativity of T-VF. The increase in the height of the T-wave in VL is due to increase in the magnitude of the positive potential developed at the left shoulder, while the deep inversion of T-VF is due to increase in the magnitude of negative potential developed at the left hindlimb. Because the left forelimb is initially positive with respect to the left hindlimb, the beam in lead III is initially deflected downward, inscribing an inverted T-wave (fig. 13). Conversely, decelerating the repolarization of this region by cooling results in increased negativity of T-VL, but in increased positivity of T-



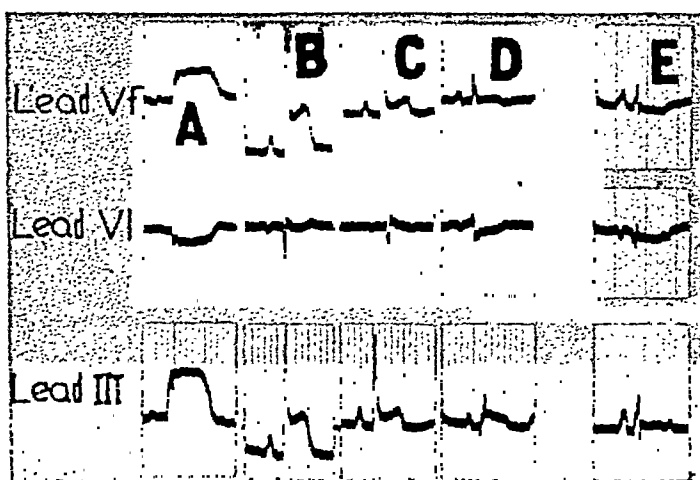


Fig. 12. FIVE DIFFERENT COMBINATIONS of ST-segment deviations in VF and VL that produce ST-segment elevation in lead III. A. 5.6 K. male dog, Aug. 21, 1947. M/5 KCl applied to segment 6, fig. 2B. The ST-segment is sharply elevated in VF, sharply depressed in VL and sharply elevated in lead III. B. 6.7 K. male dog, Sept. 11, 1947. M/5 KCl applied to segment 9, fig. 2B. The ST-segment is sharply elevated in VF, isoelectric in VL, and sharply elevated in lead III. C. 6.7 K. male dog, Sept. 11, 1947. M/5 KCl applied to segment 10, fig. 2B. The ST-segment is moderately elevated in both VF and VL, but to a greater extent in VF. ST-III is moderately elevated. D. 8.7 K. male dog, Aug. 7, 1947. M/5 KCl applied to segment 7, fig. 2B. The ST-segment is isoelectric in VF, moderately depressed in VL, and moderately elevated in lead III. E. 7.2 K. male dog, May 18, 1947. M/5 KCl applied to segment 8, fig. 2B. The ST-segment is slightly depressed in both VF and VL, but to a greater extent in VL. ST-III shows slight elevation. Control records are not exhibited, but all leads showed isoelectric ST-segments.

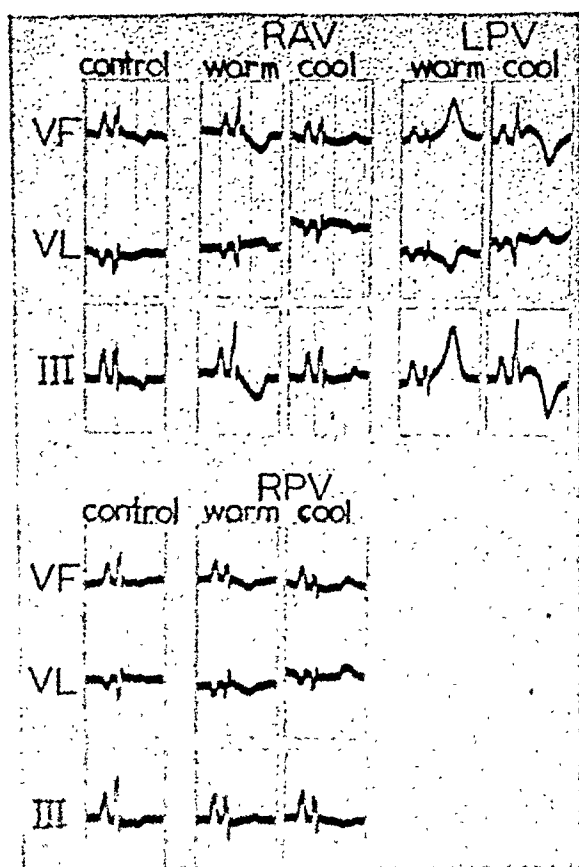


Fig. 13. CHANGES IN THE T-WAVE in leads VF, VL and III following heating and cooling the heart surface. 7.2 K. male dog, May 18, 1948. RAV = right anterior ventricle; LPV = left posterior ventricle; RPV = right posterior ventricle. Warming RAV makes T-VF more negative, T-VL more positive, and T-III sharply inverted. Cooling RAV makes T-VF more positive, T-VL more negative, and T-III sharply upright. Warming LPV makes T-VF more positive, T-VL more negative, and T-III sharply upright. Cooling LPV makes T-VF more negative, T-VL more positive, and T-III deeply inverted. Warming RPV makes both T-VF and T-VL more negative. T-III shows only moderate change. Cooling RPV makes both T-VF and T-VL more positive. T-III shows only slight change. Changes in the QRS complex are noted, and will be discussed elsewhere.

VF. Since the left forelimb is initially negative with respect to the left hindlimb during the inscription of the T-wave, the beam in lead III is initially deflected upward, inscribing an upright T-III (fig. 13). Similar treatment of the left apex and posterior left ventricle with heat and cold results in changes in T-VL, T-VF, and T-III of opposite nature to those described above following treatment of the anterior right ventricle (fig. 13).

The above experimental findings all lead to the same conclusion, namely, that depolarization of the anterior right ventricle results in the development of negativity at the left forelimb relative to the left hindlimb, while depolarization of the left apex and lower two-thirds of the posterior left ventricle results in the development of positivity at the left forelimb relative to the left hindlimb. Since in each case the potentials at the two extremities are of opposite electrical sign, lead III—recording the difference in potential between the two extremities—exhibits maximal upward or downward deflections of the beam respectively following the depolarization of these regions of the heart.

*B. Regions of the Heart in Which Depolarization Results in Deflections of the Beam in Lead III of Lesser Magnitude Than Those Described in Section A*

Upward movements of the beam in lead III also occur when regions of the heart which border immediately upon the upper half of the anterior right ventricle become activated, while downward deflections of the beam in lead III also occur when regions of the heart which border immediately upon the left apex and lower two thirds of the posterior left ventricle become activated, but these deflections are of lesser magnitude than those described above in Section A. Study of ST-segment displacements in leads VL, VF and III resulting from experimental injury to these regions furnishes the explanation for these observations.

1. M/5 KCl solution applied to segment 2, figure 2B, produces no displacement of the ST-segment in VF, but upward displacement of the ST-segment in VL, indicating that during diastole the left forelimb is negative relative to the left hindlimb. The diastolic baseline in lead III is therefore displaced upward and an ST-segment depression is inscribed (fig. 11D).

2. M/5 KCl solution applied to segment 3, figure 2B, produces slight upward displacement of the ST-segment in both VF and VL, but the degree of upward displacement is somewhat greater in VL than in VF, indicating that during diastole the left forelimb is negative relative to the left hindlimb. The diastolic baseline in lead III is, therefore, displaced upward, and a minimal ST-segment depression results (fig. 11E).

3. M/5 KCl solution applied to segment 4, figure 2B, produces downward displacement of the ST-segment in VF but no displacement of ST-VL, indicating that the left forelimb is negative relative to the left hindlimb during diastole. Lead III, therefore, exhibits a depressed ST-segment (fig. 11B).

4. M/5 KCl solution applied to segment 5, figure 2B, produces depression of the ST-segment in both VF and VL, but the degree of depression is greater in VF than in VL. The left forelimb is relatively negative with respect to the left hindlimb during diastole and, therefore, lead III exhibits slight depression of the ST-segment (fig. 11C).

5. M/5 KCl solution applied to segment 7, figure 2B, produces no displacement of ST-VF and depression of ST-VL. The left forelimb is positive relative to the left hindlimb during diastole, and, therefore, lead III shows elevation of the ST-segment (fig. 12D).

6. M/5 KCl solution applied to segment 8, figure 2B, produces slight depression of the ST-segments in both VF and VL, but the degree of depression is somewhat greater in VL than in VF. The left forelimb is positive relative to the left hindlimb during diastole and lead III therefore exhibits slight ST-segment elevation (fig. 12E).

7. M/5 KCl solution applied to segment 9, figure 2B, produces elevation of the ST-segment in VF, but no change of ST-VL. The left forelimb is positive relative to the left hindlimb during diastole and lead III exhibits ST-segment elevation (fig. 12B).

8. M/5 KCl solution applied to segment 10, figure 2B, produces elevation of the ST-segment in both VF and VL, but the degree of elevation is somewhat greater in VF than in VL. Lead III, therefore, exhibits moderate elevation of the ST-segment (fig. 12C).

Thus it has been demonstrated above that the depolarization of the upper two thirds of the anterior right ventricle, the upper two thirds of the anterior septum, the upper third of the anterior left ventricle, and a small portion of the basal posterior left ventricle (segments 1-4, fig. 2B) results in the development of negativity at the left forelimb relative to the left hindlimb and thus produces upward movement of the beam in lead III. Depolarization of the left apex, the lower third of the anterior left ventricle, a portion of the right apex, and the lower two thirds of the posterior left ventricle and septum (segments 6-10, fig. 2B) results in the development of negativity at the left hindlimb relative to the left forelimb and thus produces downward movement of the beam in lead III.

More specific localization of the site of injury to the heart can be obtained from a study of ST-segment displacements in leads VF and VL than from a study of ST-segment displacements in lead III alone. Upward displacement of the ST-segment in lead III localizes the injury only to the general region of the left apex and posterior left ventricle, while the displacements in leads VF and VL help localize the injury to more specific parts of this general region. Depression of the ST-segment in lead III localizes injury only to the general region of the anterior right ventricle, while the displacements in leads VF and VL help localize the injury to more specific parts of this region.

### *C. Regions of the Heart in Which Depolarization Results in Minimal or no Deflection of the Beam in Lead III*

Exploration of the ventricular surfaces of the heart reveals that depolarization of major portions of the posterior right ventricle and the anterior left ventricle fails to produce significant deflection of the beam in lead III. It has been demonstrated above that depolarization of these segments of the heart is maximally recorded in lead I. The reasons for the electrical neutrality of these regions in lead III are revealed from study of changes occurring in leads VF and VL following injury to these segments or following alterations in their rates of repolarization.

1. *Mid-third of posterior right ventricle (segment 12, fig. 2B).* a) M/5 KCl solution applied to this region produces ST-segment depression of equal degree in both VF and VL, indicating that both the left forelimb and left hindlimb are at the same positive potential during diastole as a result of the current of injury. Since there is no difference in potential between the two extremities during diastole, lead III therefore exhibits an isoelectric ST-segment (fig. 14A).

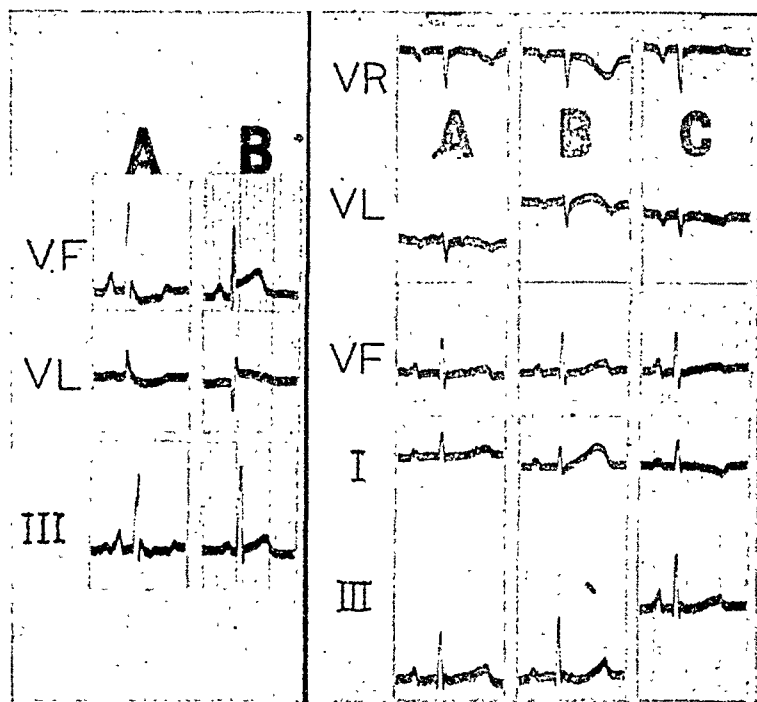


Fig. 14 (left). ST-SEGMENT DISPLACEMENTS in VF and VL that fail to produce ST-segment changes in lead III. A. 7.0 K. female dog, June 2, 1948. M/5 KCl applied to segment 12, fig. 2B. The ST-segment is depressed to the same extent in both VF and VL. The ST-segment in lead III remains isoelectric. B. 11.4 K. male dog, Aug. 15, 1947. M/5 KCl applied to segment 11, fig. 2B. The ST-segment is elevated to the same extent in both VF and VL. The ST-segment in lead III remains isoelectric. Controls showed isoelectric ST-segments in all leads, but are not exhibited.

Fig. 15 (right). CHANGES IN THE T-WAVE in leads VR, VL, VF, I, and III following heating and cooling the anterior left ventricle. 6.1 K. male dog, June 3, 1948. A = control; B = warming; C = cooling. Warming produces increased negativity of T-VR, but little change in T-VL and T-VF. T-I becomes more upright while T-III shows minimal change. Cooling produces upright T-VR, but little change in T-VF and T-VL. T-I becomes inverted while T-III remains essentially unaltered.

b) Acceleration of repolarization of this segment by heat makes the T-wave become more negative in both VF and VL. Because the degree of change in each of these leads tends to be of equal magnitude, the T-wave in lead III exhibits only moderate change in its configuration following the application of heat to this region (fig. 13). When the repolarization of this region is delayed by cooling it, both T-VF and T-VL become more positive. Because the degree of change in the T-wave in each of these leads tends to be similar, lead III fails to exhibit any significant change in T-wave configuration (fig. 13).

2. *Mid-third of anterior left ventricle (segment 11, fig. 2B).* M/5 KCl solution applied to this segment produces slight ST-segment displacement upward of equal degree in both VF and VL. Since the potentials which exist during diastole at both

the left forelimb and left hindlimb are of the same electrical sign and magnitude, lead III exhibits no displacement of the ST-segment (fig. 14B). These changes occur in VF and VL when the injury involves chiefly that portion of the mid-third of the anterior left ventricle which lies in the proximal zones of both of these leads (segment labelled *DPP*, fig. 1).

3. *Major portion of anterior left ventricle (segments 1-3, 6, 9-11, fig. 2B).* a) M/5 KCl solution applied over the entire surface of the anterior left ventricle may also produce no appreciable displacement of the ST-segment in either VF or VL, and as a consequence there will be no change in the ST-segment in lead III (fig. 9B). The explanation for this phenomenon can be understood by referring to figure 1 in which it is seen that the upper portion of the anterior left ventricle lies in the proximal zone of VL but in the distal zone of VF while the lower portion of the anterior left ventricle lies in the distal zone of VL, but in the proximal zone of VF. Injury with potassium which tends to involve equal portions of both the proximal and distal zones of both VF and VL will result in an isoelectric ST-segment in both VF and VL since the algebraic summation of the injury potentials derived from the proximal and distal zones of each of these leads will tend to approximate zero. Although no change may occur in ST-VF and ST-VL following such treatment of the anterior left ventricle, the ST-segment in lead VR is displaced downward because the injured area consists chiefly of a part of the distal zone of this lead. Lead I, therefore, exhibits definite ST-segment elevation (fig. 9C, D).

Thus it can be seen that the greater the extent of anterior left ventricular injury the more likely it is that the algebraic summation of injury potentials developed at both the left forelimb and left hindlimb will approximate zero and that no displacement of the ST-segment will occur in leads VF, VL, and III.

b) Heating and cooling the entire anterior left ventricle may also fail to produce any changes in the T-wave in lead III, for reasons similar to those given above for the lack of ST-segment deviation following injury to this region. If the area of the anterior left ventricle treated by heat or cold is of such magnitude that equal portions of the proximal and distal zones of both leads VF and VL are involved, the algebraic summation of the opposite effects which result from treatment of both zones in each lead will tend to approximate zero. The T-wave in both VF and VL would, therefore, tend to remain unaltered, and as a result T-III will remain unchanged. Although T-III remains essentially unchanged following alteration in the rate of repolarization of the anterior left ventricle, the T-wave in lead I shows marked change because the region treated consists chiefly of a portion of the distal zone of lead VR (fig. 15).

#### SUMMARY AND CONCLUSIONS

The present study of the nature of lead III by analysis of simultaneously recorded 'unipolar' extremity leads VF and VL permits the following conclusions:

1. The five possible combinations of potentials at the left forelimb and the left hindlimb that could account theoretically for upward movement of the beam in lead III and the five possible combinations of potentials at these extremities that could account theoretically for downward movement of the beam in this lead have been

shown to exist experimentally and each combination can be related to the depolarization of a specific ventricular region.

2. It is only when the site of preponderant depolarization lies within the left apex and/or the posterior left ventricle that downward deflection of the beam occurs in lead III since only depolarization of these segments results in the development of potentials at the left forelimb which are positive relative to those developed at the left hindlimb. If a portion of this region is activated in advance of the anterior right ventricle, the first deflection of the beam will be downward, inscribing a Q-wave in this lead. Later excitation of this region will again result in downward deflection of the beam inscribing the downstroke of the R- and S-waves in this lead.

3. It is only when the site of preponderant depolarization lies within the anterior right ventricle and the upper third of the anterior left ventricle that upward deflection of the beam occurs in lead III since only depolarization in these regions gives rise to potentials at the left forelimb which are negative relative to those developed at the left hindlimb. Early activation of these regions will result in upward deflection of the beam inscribing the upstroke of the Q- and R-waves in this lead. If a portion of these regions is the last site to become depolarized, the beam will again be deflected upwards, inscribing the upstroke of the S-wave.

4. No deflections of the beam take place in lead III when the major portion of the posterior right ventricle and the mid-third of the anterior left ventricle become depolarized because such depolarization fails to result in the development of any difference in potential between the left forelimb and the left hindlimb.

5. An isopotential ST-segment in lead III can occur when any of the following conditions exist: *a*) no current of injury is present; *b*) injury is present in the mid-third of the posterior right ventricle and/or the mid-third of the anterior left ventricle or injury involves the entire anterior left ventricle; *c*) repolarization has not yet begun in the anterior right and posterior left ventricles; *d*) the anterior right and posterior left ventricles are repolarizing at the same rate, thus producing potentials at the left fore- and hindlimbs of the same magnitude but of opposite electrical sign so that the net potential at each of these extremities is zero.

6. More specific localization of injury to the heart is possible from study of ST-segment displacements in leads VF and VL than from a study of ST-segment displacement in lead III alone. ST-segment elevation in lead III localizes the injury only to the general region of the left apex or lower two thirds of the posterior left ventricle, while the ST-segment displacements in leads VF and VL help localize the injury to more specific parts of these regions. ST-segment depression in lead III localizes injury only to the general region of the anterior right ventricle, while the displacements of the ST-segments in leads VF and VL help localize the injury to more specific parts of this region.

7. When the anterior right ventricle repolarizes in advance of the posterior left ventricle, the T-wave is inverted in lead III because of the development of initial relative positivity at the left forelimb. Conversely, when the posterior left ventricle and left apex repolarize in advance of the anterior right ventricle, the T-wave in lead III is upright because of the development of initial relative negativity at the left forelimb. The repolarization of the posterior right ventricle and the anterior left

ventricle contributes little or nothing to the configuration of the T-wave in lead III because the repolarization of each of these areas tends to produce simultaneous potentials of the same electrical sign and magnitude at the left fore- and hindlimbs.

These conclusions as to the derivation of lead III in the dog correspond closely with the findings of Hoff, Nahum, and Kaufman (2-5) on the nature of the various components of the ventricular complex in this lead. These authors described lead III as the algebraic summation of potentials derived mainly from the depolarization of the anterior right and posterior left ventricles. They found that depolarization of the anterior left and posterior right ventricles was not represented in lead III. The present study has more exactly delimited the electrically active as well as the electrically neutral areas for lead III, and has established the basis for the presence or absence of electrical representation of the various regions of the heart in lead III.

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# RELATIVE DISTRIBUTION OF CARDIAC OUTPUT IN ACUTE HYPOXEMIA<sup>1</sup>

M. FELDMAN, JR., S. RODBARD AND L. N. KATZ

*From the Cardiovascular Department,<sup>2</sup> Medical Research Institute, Michael Reese Hospital*

CHICAGO, ILLINOIS

THE induction of acute hypoxemia produces a rapid sequence of cardiovascular changes which follow a fairly uniform pattern. It has been shown that the cardiac output and the blood pressure are increased in hypoxemia (1-4). However little information is available on the moment to moment alterations in the circulation of blood during the induction of acute hypoxemia and its readjustment following recovery from the hypoxemic state. Likewise, few data are available on the possible changes in the differential distribution of blood flow during the acute hypoxemic and post-hypoxemic periods. Such data would be valuable in interpreting the hemodynamic changes which occur. In the present study we have attempted to estimate the changes in cardiac output during the various phases of hypoxemia and reoxygenation, and to some extent to determine the relative distribution of the blood flow between the upper and lower parts of the body. This was done by estimating cardiac output on the basis of blood flow through the superior or inferior vena cava.

## METHODS

Blood flow was measured in the inferior or superior vena cava with a modified Ludwig stromuhr in vagotomized, heparinized, open-chested dogs, anesthetized with sodium pentobarbital (25 mg/kilo). Flow was determined by the time required for the displacement of 40 cc. volumes in the stromuhr, which required three seconds to 'infinity' (no perceptible flow). The blood flow in the superior vena cava was measured in 7 dogs in 38 periods of hypoxemia and recovery and the flow in the inferior vena cava in 6 dogs in 40 periods. Artificial respiration in open-chested animals assured a controlled respiration throughout the experiment without the intervention of factors related to respiratory failure.

Acute hypoxemia was produced for periods of 2 to 3½ minutes by the substitution of 100 per cent nitrogen for air breathing; reoxygenation was accomplished by returning the animal to air breathing. Serial readings of the blood flow were taken during a control period and repeated each 15 to 30 seconds during the period of hypoxemia and reoxygenation. Mean femoral blood pressures were recorded with a mercury manometer on an ink writing kymograph. The circulation time was estimated in 10 trials on 5 dogs using the acetylcholine method of Wilburne *et al.* (6). This technique measures the time required for circulation of blood from the site of injection to the nodal tissue of the heart, as indicated by a blocked beat.

Received for publication July 2, 1948.

<sup>1</sup> Aided by the A. D. Nast Fund for Cardiovascular Research.

<sup>2</sup> The department is supported in part by the Michael Reese Research Foundation.



## RESULTS

The changes observed in the hypoxemic and post-hypoxemic periods are illustrated in figure 1 by a graph of typical data obtained in a series of experiments in 2 dogs in which the conditions of anesthesia and blood pressure were similar. Data on the experiments are given in tables 1 and 2.

1. *Effect of acute hypoxemia.* The induction of severe progressive hypoxemia almost always produced a rise in blood pressure ranging from 9 to 44 mm. Hg, which usually began about 30 seconds after the onset of nitrogen breathing. The pressure then leveled off and began to fall rapidly to shock levels. During the first two

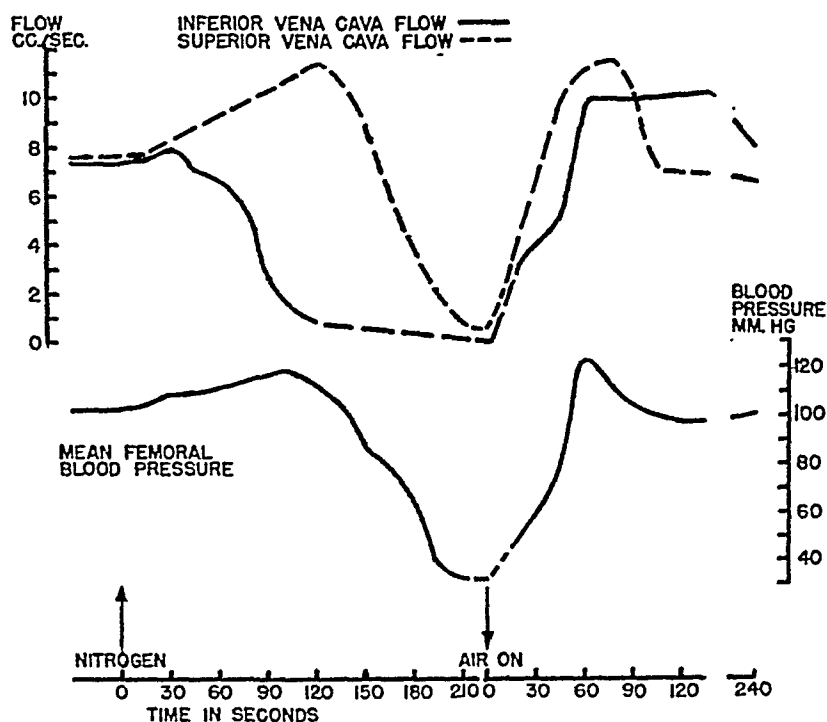


Fig. 1. THE ABOVE GRAPH REPRESENTS the average data obtained during 14 acute hypoxemic and reoxygenation periods in 2 dogs in which conditions of anesthesia and blood pressure were similar. The mean femoral blood pressure shown above is that of the dog in which the superior vena cava flow was measured. The blood pressure changes of the other dog were similar to those shown above.

minutes of the hypoxemic pressor phase a marked acceleration of blood flow up to double the control value was observed in the superior vena cava (fig. 1, table 2).

On the other hand the flow in the inferior vena cava showed only a slight or moderate increase in flow during the early part of this period. Within approximately 75 seconds after the onset of the hypoxemic period, the flow through the inferior vena cava diminished suddenly and markedly although the blood pressure was still above control values. Thirty seconds later no perceptible flow was measurable in this circuit. At this time of markedly diminished flow in the inferior vena cava, the flow through the superior vena cava was usually at or above control values and the blood pressure averaged 87 mm. Hg compared to 93 mm. Hg for the average control value (fig. 1, table 1).

The superior vena cava flow usually (24 of 38 trials) continued at or above the

control flow rates after the blood pressure had fallen below its control level. As the hypoxemia continued, both the blood pressure and superior vena cava flow fell progressively in all instances. In 19 trials the superior vena cava flow stopped almost completely during the late depressor phase of acute hypoxemia, when the blood pressure had fallen to an average of 40 mm Hg.

TABLE 1. FLOW IN THE INFERIOR VENA CAVA

TIME	DOG NO.											
	IVC 1		IVC 2		IVC 3		IVC 4		IVC 5		IVC 6	
	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow
sec.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.
-5	77	14.8	101	8.7	75	7.0	108	7.4	94	7.0	94	6.6
Nitrogen												
0	79	14.3	105	8.7	75	6.7	111	7.4	95	6.7	93	6.1
15	84	15.3	123	9.5	75	7.4	114	7.5	95	6.5	99	7.1
30	94	16.6	147	6.2	79	7.6	130	8.0	101	7.8	103	7.6
45	105	17.4	131	4.2	85	6.7	135	7.0	125	9.9		
60	119	19.0	98	1.3	96	6.0	141	6.6	136	7.1	109	7.6
75	120	17.4	103	0.4	95	6.1	146	5.9	108	1.5	105	7.7
90	108	9.8	air	air	105	5.0	125	2.7	air	air	99	6.1
105	84	4.3			98	1.9					90	5.5
120	64	0.5			83	1.1	98	0.7			74	2.5
Air												
15	53	2.8	124	6.3	87	3.6	88	3.4	89	0.7	90	5.2
30									98	5.2	144	9.5
45					151	9.3	129	5.2				
60	127	22.2	115	10.0			156	10	99	7.3	169	11.1
75					165	9.3						
90	112	28.8			134	8.7	140	10	98	8.5	160	10
120	93	25.2			107	8.3	124	10.3	99	8.7	139	8.2
150	90	21.0			90	7.4	122	10	98	8.5	127	7.7
180	86	20.2					117	9.5	96	7.8	115	8.0
240	77	16.7	96	8.0	77	7.1	108	8.0	89	7.3	96	7.4

0 seconds represents the onset of nitrogen breathing. *Air* indicates the time of cessation of nitrogen and the return to air breathing. The data represent average results of several observations on each dog.

When acetylcholine was injected into the femoral vein during the late depressor phase of hypoxemia the average circulation time was greatly increased, averaging 79 seconds in 10 trials as compared to control values averaging 11 seconds. However, this long delay in circulation time was not apparent when the acetylcholine was injected into the heart or into the superior vena cava instead of the femoral vein. The marked slowing of circulation rate in the inferior vena cava and not in the superior vena cava during the depressor phase of acute hypoxemia is in accord with our results obtained with the stromuhr. Direct observation of the heart revealed marked cardiac dilatation, occasional arrhythmias, and slowing during the later stages of the depressor phase of hypoxemia.

2. *Effect of reoxygenation.* With the resumption of air breathing a sudden marked increase in flow through both vena cavae was noted and this was followed almost at once by a rise in blood pressure. In most instances a marked increase in flow was observed even before the blood pressure had risen significantly. Later during this reoxygenation period the blood pressure often reached levels much higher

TABLE 2. FLOW IN THE SUPERIOR VENA CAVA

TIME	DOG. NO.													
	SVC 1		SVC 2		SVC 3		SVC 4		SVC 5		SVC 6		SVC 7	
	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow	BP	Flow
sec.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.	mm. Hg	cc/min.
-5	82	8.8	112	8.0	88	7.0	80	5.7	64	3.7	88	4.0		7.7
Nitrogen														
0	82	8.8	108	8.8	84	6.6	82	5.0	64	3.6	88	3.8	102	7.7
15			109	8.0	87	6.3			67	4.4	88	3.4	104	7.7
30	83	10.0	110	10.0			117	6.6	78	5.0			109	8.3
45	85	12.2			95	9.7			74	5.7	116	7.3	108	8.7
60	90	13.2	112	10.0	98	10.0	106	2.7	86	6.8	118	10.0	110	9.3
75	91	16.0	113	13.3	102	10.8			103	10.0	118	16.0		
90	75	11.0	108	13.3	98	11.4	55	0.9	88	8.0	101	13.3	117	10.3
105	52	6.9	100	13.3	83	9.9			87	8.0	89	13.3	118	11.4
120	40	2.7	82	11.4	63	5.3	air	air	72	4.0	70	11.4	112	12.5
135	35	1.1	68	8.0					42	1.3	60	8.0	106	12.9
150	air	air	51	5.0	44	2.6			43	0	51	7.3	88	9.3
165			28	0	42	2.5			air	air	47	5.7	78	6.3
180			air	air	39	1.7					48	5.0	67	4.4
195					air	air					50	4.4	39	2.2
210											air	air	33	0.9
Air														
15					67	4.4					58	6.6	45	3.6
30							88	5.7						
45					120	11.4					110	10.0	81	10.0
60													123	11.7
75	73	12.5	45	10.0					47	4.0	118	7.3		
90	101	14.3	110	13.3	99	8.3	72	7.3					105	7.0
120			170	13.3					83	5.0	121	7.3	96	7.0
240	77	8.4	120	8.0		7.1		5.7	60	3.8		5.7		6.6

See footnote to table 1.

than in the early hypoxemic phase. The pressor effect was soon dissipated and the blood pressure and blood flow through both vena cavae returned to control levels approximately four minutes after resumption of air breathing.

#### DISCUSSION

These results suggest that the first effect of hypoxemia was to produce an increase in the rate of return blood flow to the heart and thus an increase in the cardiac output. This was accompanied by a rise in blood pressure. The augmented blood flow

would act to increase the perfusion of the tissues and would normally compensate for the hypoxemia. As the hypoxemia continued, the flow in the inferior vena cava began to fall and was markedly reduced even though the blood pressure and superior vena cava flow were still above control values. It is apparent that at this time, a preferential redistribution of blood to the vital head region occurs. Continuance of the hypoxemia results in a fall in the superior vena cava flow as well as in the inferior vena cava flow, and this is furthered by progressive cardiac failure.

It is possible that our mode of flow measurement does not give an entirely satisfactory picture of the cardiac output, since we are measuring the venous return to the heart exclusive of the azygos flow and the important coronary flow. The pooling of blood in the periphery as a result of vasodilatation would unquestionably introduce deviations which we could not determine with our technique. Nevertheless our technique does offer an index of the cardiac output which appears to be in accord with our findings on the changes in circulation time during hypoxia. The flow of blood through the coronary circuit which we could not measure, appears to play an important rôle in the mechanism of recovery.

With reoxygenation the blood flow increases almost at once and this is quickly followed by a marked rise in arterial pressure. These changes are made possible by rapid recovery of the heart as the result of an adequate oxygen supply, as well as by vasoconstriction in the periphery. At this time endogenous pressor materials produced during the hypoxemic period probably contribute to the blood pressure rise (6).

Our experiments suggest that some of the blood pressure effects seen in acute hypoxemia and in the reoxygenation phase are related to the marked changes in cardiac output. These output changes are due most likely to vasodilating effects induced by tissue hypoxia upon the peripheral vessels. As a consequence a redistribution of the circulating blood flow occurs. There can be little doubt that central nervous mechanisms as well as humoral factors contribute to these effects. Our results are in accord with other findings that the cardiac output is a function of the tissue oxygen requirements and that when tissue hypoxia exists the cardiac output will rise as a compensatory mechanism. When the lack of oxygen becomes extreme the heart and central nervous system begin to weaken and the cardiac output falls probably because of heart failure. With resaturation of the blood and tissues with oxygen, the chain of events is reversed and cardiac output rapidly returns to its basal level with a transitory overswing.

With our technique we have been able to perfuse tissues with hypoxemic blood without a concomitant hypercapnia. Consequently, the large changes in blood flow occurring under these conditions are not explained by alterations in  $\text{CO}_2$  content. Reoxygenation of the blood results in an increased flow similar to that seen in local reactive hyperemia first described by Lewis (7). Thus it would appear that a large part of the stimulus for this reactive hyperemia throughout the body must be due directly to oxygen lack and not entirely to the production during the hypoxemic period of other easily diffusible substances which can be lost in the lungs or quickly disposed of by the liver.

This is indicated by the remarkably rapid recovery of the severely hypoxemic

heart upon resumption of air breathing. During the late phases of the hypoxemic period, the cardiac output as measured by the flow in both venae cavae was extremely low. The heart could be seen to be dilated and beating feebly. Nevertheless, within a few seconds after the reestablishment of air breathing the heart was seen to beat vigorously again, cardiac output markedly improved and the blood pressure rose rapidly. The fact that oxygenated blood from the lungs is able to reach the coronary circulation so rapidly and in sufficient amounts to effect such a rapid recovery, indicates that some residual flow must have been continuing through the heart-lung-coronary circulation. Such blood would include that being returned through the coronary vessels and perhaps some via the azygous veins. The flowmeter offers some resistance to flow and if only a small amount of blood were being pumped by the heart this blood could easily bypass the flowmeter and pass through non-cannulated parallel circuits which offer less resistance.

#### SUMMARY

The return of blood flow to the heart through the superior or the inferior vena cava was measured in 13 dogs subjected to acute hypoxia produced by nitrogen breathing. With the onset of acute hypoxemia the blood flow and pressure increased. After about 75 seconds the flow through the inferior vena cava fell rapidly to nearly zero levels although the superior vena cava flow remained above normal. As the hypoxemia progressed, the superior vena cava flow also diminished. At this time the blood pressure was low and the heart was seen to be dilated and beating slowly. Reoxygenation resulted in an immediate recovery of cardiac function of blood flow in both circuits and of the blood pressure which returned to the previous control levels within a few minutes with a temporary overswing.

The mechanisms of some of the hemodynamic changes occurring during the induction of and recovery from acute hypoxemia are discussed.

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# NATURE OF THE TWO PRESSOR RESPONSES TO ACUTE HYPOXEMIA WITH SOME OBSERVATIONS ON THE RÔLE OF THE ADRENALS IN HYPOXIA<sup>1</sup>

A. VAN LOO<sup>2</sup>, A. SURTSHIN<sup>3</sup> AND L. N. KATZ

*From the Cardiovascular Department<sup>4</sup>, Medical Research Institute, Michael Reese Hospital*

CHICAGO, ILLINOIS

WE HAVE described, in detail, the responses of the blood pressure and heart rate to acute severe hypoxemia (1). During such a hypoxemia, produced in open-chested dogs by breathing pure nitrogen, the rise in pressure (hypoxemic pressor phase) is followed by a fall (hypoxemic depressor phase). The depressor phase is progressive ending in death if nitrogen breathing is maintained. However, when air is substituted for nitrogen, a second pressor effect (post-hypoxemic pressor phase) is seen which is frequently greater in magnitude than the primary pressor effect.

We have shown that there is a relationship between the height of the post-hypoxemic rise and the duration of the hypoxemic depressor phase. This relationship suggests that, during the depressor phase, substances were elaborated which could not exert their pressor effect or be quickly destroyed in the absence of oxygen. With reoxygenation, the pressor action of these substances becomes manifest. This concept was strengthened by our finding that injected epinephrine acts similarly, with no pressor effect during the late hypoxemic depressor phase and a pressor action as soon as air breathing is resumed. This led us to conclude that the pressor material liberated during the hypoxemic phase was epinephrine-like in action. The nature of the hypoxemic pressor phase was not indicated by our experiments. The present study was undertaken to test these concepts and to define the rôle of the adrenal gland in these pressor response to acute hypoxemia.

## METHODS

Eighty-three experiments were performed upon 12 open-chested vagotomized dogs, anesthetized with pentobarbital sodium (25 mg/kg.). The thorax was opened in the fifth or sixth intercostal space, the adjacent ribs were maintained widely retracted and the thin mediastinal septum was destroyed. Hypoxemia was produced by replacing the respired air with nitrogen (1).

Six of the 12 animals were adrenalectomized and the other 6 were prepared by a modification of the technique described by Bouckaert and Van Loo (2). Our technique involves elimination of the right adrenal gland from the circulation by

Received for publication July 12, 1948.

<sup>1</sup> Aided by the A. D. Nast Fund for Cardiovascular Research.

<sup>2</sup> Fellow of the Belgian American Educational Foundation.

<sup>3</sup> Herbert G. Mayer Fellow, now at Lahey Clinic, Boston, Massachusetts.

<sup>4</sup> The department is supported in part by the Michael Reese Research Foundation.

ligature and the isolation of the venous drainage from the intact innervated left adrenal gland. All veins tributary to the left adrenal gland drainage were tied off, leaving undisturbed only the two major venous trunks, the adreno-caval and the lumbo-adrenal veins. The former was maintained intact, being cleared from the surrounding tissue so that it could be clamped as desired. The lumbo-adrenal vein was cannulated a short distance below the point where it entered the capsule of the adrenal gland, with the cannula opening toward the hilus. The cannula was kept open by means of a slow saline drip under constant pressure. During this time, the venous output of the left adrenal gland drained, via the adreno-caval vein, into the inferior vena cava and thus was in the circulation of the animal. When it was desired to eliminate the effects of the adrenal from the experiment, the adreno-caval vein was clamped and gentle suction was applied by means of a syringe attached to the cannula via a three-way stopcock and the experiment was repeated. In this way the entire venous outflow from the left adrenal gland could be collected and measured, while the responses of the animal with the adrenal out of the circulation were being recorded. To test the pressor effect of the blood so collected upon the same animal, the clamp on the adreno-caval vein was removed and the collected blood was reinjected within about 10 secs. Blood pressures were recorded continuously upon a kymograph with a mercury manometer.

## RESULTS

1. *Hypoxemic Pressor Phase.* The pressor response during the hypoxemic period is usually definitely reduced by the diversion of adrenal blood from the circulation. This is clearly illustrated in figure 1. While this relationship does not appear in every instance and at times the responses are similar with the gland in and out of the circulation, it is revealed by a comparison of the averages of the absolute rises in pressure seen in two types of experiments. These figures, given in table 1, show that on the average the absolute level attained is only slightly higher when the adrenal secretion participates in the response. Thus the average response in the intact animal was a rise of 33 mm. Hg, while the average response in the animal with the adrenal out of the circulation was 27 mm. Hg. Experiments with the adrenal in and out of the circulation were conducted alternatively to compensate for changes in the control blood pressure level and general condition of the dog as the experiment proceeded. The time at which the hypoxemic peak was attained was the same in both instances.

2. *Changes Occurring After the Reinstitution of Air Breathing.* We have compared the post-hypoxemic pressure maxima obtained in experiments in which the adrenal venous drainage was alternatively permitted to flow into the systemic blood stream and, on the next trial, diverted into a syringe. As can be seen from a typical experiment in figure 1, there is a marked difference in the post-hypoxemic maxima obtained under these two conditions. In figure 1A, it will be seen that when the adrenal was participating, the resumption of air breathing was followed by a pressure increase of 54 mm. Hg above the reference level. In figure 1B, the experiment was repeated with the adrenal drainage diverted from the circulation and being collected in a syringe. Following resumption of air breathing in this case the pressure returned

to approximately the reference level. After the pressure had leveled off close to the control blood pressure level, the collected blood was reinjected into the blood stream. A second rise was thus produced with the blood pressure level now reaching an aver-

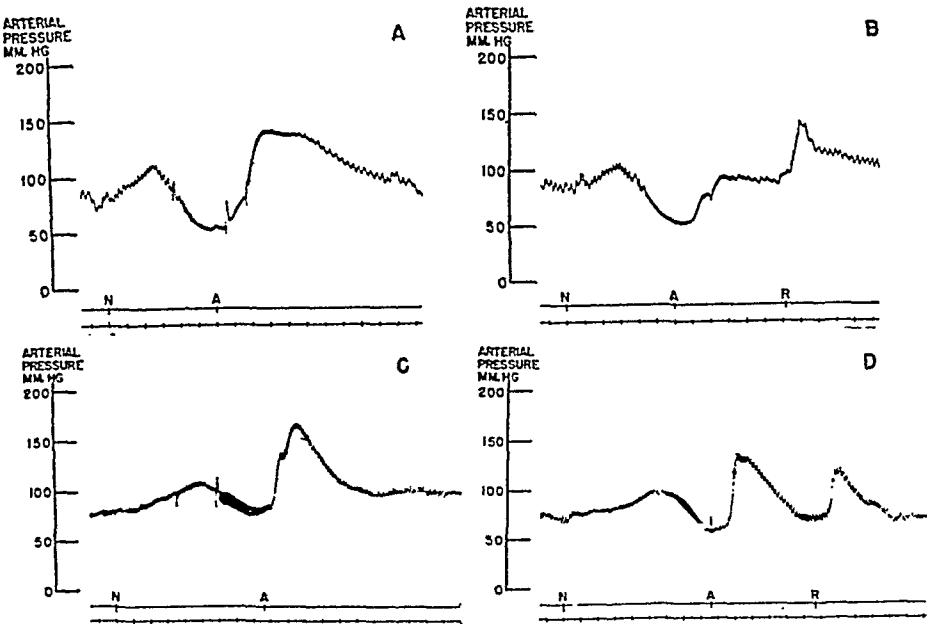


Fig. 1. BLOOD PRESSURE IN FEMORAL ARTERY. Time in 15-sec. intervals. *N* indicates time of onset of nitrogen breathing and *A*, onset of reinstitution of air-breathing. Segments *A* and *B* are from dog 1; *C* and *D* from dog 3. Segments *A* and *C* serve as controls; the adrenal blood is draining into the systemic circulation. In segments *B* and *D*, the adrenal venous blood is diverted from the circulation beginning with the onset of nitrogen breathing and is reinjected into the systemic circulation at *R*. Discussed in text.

TABLE 1. ARTERIAL PRESSURE RESPONSE DURING NITROGEN BREATHING

DOG NO.	ADRENAL GLAND IN CIRCULATION			ADRENAL GLAND NOT IN CIRCULATION		
	No. of exper.	Av. max. blood pressure level	Av. blood pressure rise above reference level	No. of exper.	Av. max. blood pressure level	Av. blood pressure rise above reference level
		mm. Hg	mm. Hg		mm. Hg	mm. Hg
1	6	120	25	4	111	11
2	11	89	27	7	77	34
3	2	127	29	2	104	16
4	4	126	30	4	114	23
5	2	135	48	2	115	32
6	5	130	54	5	114	39

age of 54 mm. Hg above the reference level. Thus, the rôle of the adrenal secretion in the production of the post-hypoxemic rise is clearly portrayed.

Data in table 2 compare the rise in arterial pressure seen in other alternate experiments with the adrenal gland in and out of the circulation. Elimination of the adrenal gland from the circulation is seen to result in a markedly diminished rise in the arterial pressure after the reinstitution of air breathing. Thus the average



TABLE 2. ARTERIAL PRESSURE RESPONSE ON RESUMING AIR BREATHING FOLLOWING NITROGEN BREATHING

DOG NO.	N <sub>2</sub> BREATHING	ADRENAL IN CIRCULATION		ADRENAL OUT OF CIRCULATION		
		Control blood pressure	Rise above reference level	Control blood pressure	Rise above reference level	Pressor activity of blood
	<i>sec.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
1	80	104	12	98	0	—
	75	104	12	104	-12	20
	90	90	54	88	+2	52
	90	72	64	88	0	38
2	155	70	70	48	54	50
	155	56	30	44	30	32
	205	56	44	50	46	44
	205	36	73	36	18	22
	240	42	50	42	22	12
3	120	114	98	98	46	43
	130	82	88	80	58	48
4	150	92	86	92	24	48
	120	112	16	98	6	32
	120	94	20	92	20	28
	120	84	38	84	24	16
5	180	80	114	62	60	66
	180	94	58	94	42	46
6	120	92	110	74	42	42
	120	80	42	80	-12	30
	120	74	2	78	-16	20
	120	66	-6	72	-16	32
	120	70	-16	72	-28	12

TABLE 3. RELATION OF DURATION OF HYPOXEMIC PERIOD TO BLOOD PRESSURE RESPONSE IN A TYPICAL BILATERALLY ADRENALECTOMIZED DOG

PER. OF N <sub>2</sub> BREATHING	CONTROL BLOOD PRESSURE	HYPOXEMIC PEAK	HYPOXEMIC RISE <sup>1</sup>	POST-HYPOXEMIC PEAK	POST-HYPOXEMIC RISE <sup>1</sup>
<i>sec.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
90	82	104	22	70	-12
120	102	116	14	88	14
150	84	104	20	126	42
180	86	104	18	144	58
210	84	106	22	158	74
240	78	102	24	158	80
300 <sup>2</sup>	70	102	32	78	8

<sup>1</sup> Above the reference level.<sup>2</sup> This trial was followed by death of the animal.

response in the intact animal was a rise of 46 mm. Hg, while in the animal with the adrenals out of the circulation the rise is only 23 mm. Hg. The reinjection of the collected blood caused an average rise of 33 mm. Hg, more than enough to account

for the deficit in the physiologically adrenalectomized animal. However, that extra-adrenal factors may also operate by themselves to produce a post-hypoxemic rise above the reference level is shown in table 3.

The 6 animals which were bilaterally adrenalectomized were subjected to varying periods of nitrogen breathing. The responses of the arterial pressure in the hypoxemic and post-hypoxemic periods in a representative example are tabulated in table 3. It will be seen that both the absolute level of the post-hypoxemic rise and the rise as measured from the reference level appear to be related directly to the duration of the hypoxemic period.

3. *Pressor Activity of the Blood Collected During Hypoxemia.* After the post-hypoxemic peak due to extra-adrenal mechanisms was recorded, we determined the pressor activity of the blood which had been collected during the hypoxemic period. This was done in two ways in different experiments: *a*) the blood was reinjected into the animal immediately after the initial post-hypoxemic peak was reached and the response noted (fig. 1B) and *b*) the blood was reinjected at a later period as the blood pressure reached the control levels (fig. 1D). Data on pressor activity are given in table 2. The correspondence between the average absolute pressures attained during the post-hypoxemic phase when the adrenal gland was in the circulation, and the average totals obtained by adding the pressor activity of the blood to the extra-adrenal post-hypoxemic peaks is apparent.

In order to ascertain when the greatest amount of pressor activity appeared in the blood of the adrenal drainage, during the period of hypoxemia, the blood in 10 experiments was collected in several samples, each of which was later separately tested for its pressor activity. The rate of blood flow from the adrenal in cc/min. was also noted in order that some conclusions regarding the concentration of pressor material might be drawn. Our results show clearly that, during the period when the systemic pressure is elevated, there is a definite increase in venous drainage from the adrenal gland. Values for adrenal flow during control periods ranged from 1.5 to 4 cc/min. During the elevation of blood pressure in the hypoxemic phase the measured flow increased from 50 to 100 per cent. It was also clear that while the volume of the blood collected during the hypoxemic pressor phase was much greater than that obtained during the hypoxemic depressor phase, the pressor activity of the former blood was markedly less than that of the smaller volume obtained during the later depressor stages of nitrogen breathing. For example, during a typical experiment in which nitrogen breathing was continued for four minutes, three blood samples were collected. The first, containing 7.5 cc., was collected during the first 2 minutes (hypoxemic pressor phase), the second, containing 3.5 cc., during the next 1½ minutes (hypoxemic depressor phase) and the third, containing 2 cc., was obtained during the 50 seconds following the reinstitution of air breathing while the arterial pressure was at its lowest point during the experiment (the blood drainage from the adrenal gland at this time being still markedly hypoxemic as indicated by its color). When these samples were reinjected into the animal at approximately control blood pressure levels, the first sample produced a rise of 2 mm. Hg, the second, a rise of 14 mm. Hg and the third and smallest sample, a rise of 22 mm. Hg. Thus it is clear that the greatest production of pressor material by the adrenal gland occurs during the later stages of hypoxemia while the blood pressure is falling. It also

appears that adrenal drainage collected during the hypoxemic pressor phase shows no greater pressor activity than blood collected during control periods of air breathing.

In order to obtain further information regarding the pressor activity of adrenal drainage and its relation to adrenal flow and systemic arterial pressure, we collected the adrenal outflow in six experiments during a period of marked elevation of the arterial pressure produced by intravenous injection of 0.2 mg. epinephrine while the animal continued to breathe air. During a representative experiment, the pressure rose from a level of 40 mm. Hg to a level of 184 mm. Hg. The adrenal blood collected during the 265 seconds after injection of epinephrine totaled 14 cc. When reinjected into the animal after its return to control pressure levels, this blood produced a rise

TABLE 4. PRESSOR ACTIVITY OF ADRENAL VENOUS DRAINAGE DURING AIR BREATHING AND CHANGES IN SYSTEMIC ARTERIAL PRESSURE DURING COLLECTING PERIOD

DOG NO.	COLLECTION PER.	BLOOD PRESSURE AT BEGINNING OF COLLECTION PER.	BLOOD PRESSURE AT END OF COLLECTION PER.	AMOUNT OF BLOOD COLLECTED	BLOOD PRESSURE ATTAINED WITH REINJECTION OF COLLECTED BLOOD	PRESSOR ACTIVITY
	sec.	mm. Hg	mm. Hg	cc.	mm. Hg	mm. Hg
1	150	82	64	9	76	12
2	150	84	60	8	70	10
	155	43	32	8½	46	14
	205	40	32	8	42	10
	245	46	34	6	44	10
	660	40	26	9½	38	12
3	150	82	74	4½	84	10
	150	60	50	3	54	4
	165	88	82	7	96	14
4	120	98	86	5	96	10
5	180	144	108	9	144	36
	180	88	50	7	96	46
6	120	84	76	11	92	16
	120	78	70	10	82	12

of 2 mm. Hg. Collection of adrenal blood for an equal period of time, in this animal, before epinephrine was given yielded a volume of 6 cc. which produced a rise of 10 mm. Hg. It appears from these results that there is a decrease of pressor activity during the time of epinephrine action as compared with the controls, but apparently no primary relation between the pressor activity of adrenal drainage and its volume.

4. *Pressor Activity of the Adrenal Venous Drainage During Control Periods of Air Breathing.* Blood was collected from the adrenal gland in 14 experiments for varying periods of time after the blood pressure had become stabilized and while the animal was lying quietly during air breathing. In each of 6 animals there was a slow but steady fall averaging 14 mm. Hg while the adrenal blood was being collected. At the conclusion of the collection period, this blood was reinjected into the animal. In each of the 6 animals there was a slow, but steady fall in systemic

arterial pressure while adrenal blood was being collected. In each instance, reinjection caused a rise of arterial pressure to a level closely approximating that existing at the beginning of the collection period. Data on this point are given in table 4.

#### DISCUSSION

It has been known for some time that an animal made acutely hypoxemic will respond with a rise in the arterial pressure which, with continuing hypoxemia, then falls until the animal succumbs. If air breathing is reinstituted after hypoxemia, a post-hypoxemic rise in pressure is produced which often exceeds that of the hypoxemic elevation. The adrenal gland, the heart and the sympathetic system have been implicated in these responses. Kaya and Starling (3) maintained that the post-hypoxemic pressure augmentation resulted from an increase in contractile power of the heart caused by oxygen. Mathison (4) mentions an effect of oxygen upon the vasomotor center. Other authors (5) have maintained that there is a post-hypoxemic excitation of the sympathetic nervous system. Stavratsky (6) has reported that adrenalectomized animals on optimal ventilation show a hypoxemic rise with nitrogen breathing and also a prominent post-hypoxemic rise after oxygen breathing is resumed. As we have commented previously (1) there has been no reported quantitation of the rôle of the adrenal gland in these changes as compared with adequate controls in the same animal. On the basis of a relationship demonstrated between the hypoxemic depressor phase and the height of the post-hypoxemic rise, it was presumed that an epinephrine-like pressor material was liberated during acute hypoxemia (1). This concept is in agreement with other reported evidence (10). It was also demonstrated that epinephrine injected intravenously, during the depressor phase of acute hypoxemia, produced little or no pressor response. The data we have presented in this report demonstrate that the adrenal gland plays only a minor rôle in the production of the rise in arterial pressure during the phase of nitrogen breathing. Its rôle, however, in the production of the post-hypoxemic rise, while variable, is a major one. It is clear that the extra-adrenal factors, probably involving the sympathetics, are in themselves capable of producing a post-hypoxemic rise which may go above the level of the control pressure, but in the absence of the venous drainage from the adrenal gland, the post-hypoxemic rise is considerably diminished. However, even in its diminished form, the magnitude of the post-hypoxemic rise resulting from extra-adrenal factors is itself a function of the duration of the hypoxemic period. These data confirm our previous conclusions that in the absence of oxygen the pressor material liberated in the body during hypoxemia is powerless to act. Only as oxygen again reaches the tissues is its pressor effect revealed.

Our data also demonstrate that the greatest production of pressor material by the adrenal gland occurs in late hypoxemia when the blood pressure is falling. It is not significantly increased during the period of the hypoxemic rise (10). Since we found that the withdrawal of adrenal drainage in air-breathing leads to a drop in blood pressure, the differences seen in the hypoxemic rises with the adrenal in and out of the circulation, may be due, at least partially, to the same loss (compare table 1 with table 4). The rate of flow of adrenal venous drainage maintains a direct relationship to the level of the arterial pressure, but there is no relationship of the

pressor activity of the collected blood to the rate of flow (10). In fact, it appears that while the rate of venous drainage from the adrenal gland is high during a period of elevation of the systemic blood pressure caused by the injection of epinephrine during air breathing, the pressor activity of this blood is, if anything, lower than that collected during control periods of air-breathing. The mechanism of the apparent reduction in adrenal activity, during the pressor phase of epinephrine, is probably related either to a diminution of the sympathetic tone or to a humoral inhibition of the gland caused by circulating injected epinephrine or, to both mechanisms (11).

Our results also indicate that removal of the venous drainage of the adrenal gland from the systemic circulation is accompanied by a slow steady fall in the arterial pressure and that reinjection of the collected blood raises the arterial pressure approximately to the level of the systemic arterial pressure when the collection was begun. Thus, at least under the conditions of our experiments the adrenal appears to play a significant rôle in the regulation of the blood pressure (cf. 7 and 8). Our evidence that hypoxemia in anesthetized animals causes an increased liberation of pressor material from adrenals seemingly is opposed to other published results (9).

It is justified to conclude that the adrenals come into play in conditions of low blood pressure, as in shock and in hypoxemia, to help neutralize the ill effects of these states by raising the blood pressure and redistributing blood to vital organs.

#### SUMMARY

The factors responsible for the pressor responses during the hypoxemic phase and in the post-hypoxemic phase were investigated. In unilaterally adrenalectomized animals, diversion into a syringe of the venous blood draining from the remaining adrenal resulted in no significant change in the hypoxemic pressor phase, but diminished considerably the post-hypoxemic response. Blood collected in the phase of the falling blood pressure during nitrogen breathing had markedly more pressor activity than that collected during the earlier phases of hypoxemia. Reinjection of such collected blood immediately after the peak of the post-hypoxemic response due to extra-adrenal factors caused the pressure to rise to a level similar to that attained in the control experiment.

It is concluded that the adrenal gland plays little part in the production of the hypoxemic pressor response, but plays a major rôle in the production of the arterial pressor response after re-aeration. Pressor material liberated from the adrenal during severe hypoxemia does not exert pressor effect until tissues are reoxygenated.

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## LUNG FUNCTION STUDIES. II. THE RESPIRATORY DEAD SPACE<sup>1,2</sup>

WARD S. FOWLER<sup>3</sup>

*From the Department of Physiology and Pharmacology, Graduate School of Medicine,  
University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

CERTAIN methods of estimating pulmonary ventilatory efficiency are limited by the accuracy of the measurement of respiratory dead space (1, 2). Krogh and Lindhard measured the physiological dead space and concluded that it varied within narrow limits during changes in lung inflation (3); on the other hand, Haldane and Priestley (4) maintained that the dead space might increase as much as 800 cc. during maximal lung inflation. Though subsequent investigations (5, 6) have in general confirmed the work of Krogh and Lindhard, there still remains considerable uncertainty about the magnitude and constancy of the volume of the respiratory dead space. Within the past few years some investigators have employed a single dead space volume for different tidal volumes (1) whereas others have used two values for dead space, one for shallow and another for deeper breaths (7). The development by Lilly and Hervey (8) of the nitrogen meter, for continuous analysis of the nitrogen concentration of respired gases, has made possible the reinvestigation of this problem.

Since the terminology used by various writers is not uniform, it seems advisable to clarify the meaning of dead space. The respiratory system may be divided into those parts which serve primarily as a conducting airway and not as sites for rapid change of O<sub>2</sub> and CO<sub>2</sub> (mouth, nose, pharynx, larynx, trachea, bronchi and bronchioles) and those whose chief function is gas exchange (alveoli, alveolar sacs and atria). If a sharp separation could be made between the two, the former would be defined as the dead space and the latter as the container of alveolar air. If the dead space gas could be expelled from the respiratory tract as a bloc of gas with a sharp boundary line dividing it from the alveolar gas, its measurement would be simplified. However several factors prevent this: first, anatomical studies indicate that the boundary between conducting and exchange airway is not definite; second, diffusion occurs at this boundary area and obscures any sharp margin; third, when the gas is put into motion during expiration, some alveolar gas pushes into the dead space gas and so eliminates a square front. The latter process of expiratory gas mixing

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Received for publication June 25, 1948.

<sup>1</sup> Presented in abstract form at the annual meeting of the American Physiological Society on March 16, 1948.

<sup>2</sup> The work described in this paper was done under contract between the Medical Division, Chemical Corps, U. S. Army and the University of Pennsylvania. Under the terms of the contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the authors.

<sup>3</sup> Research Fellow of the American College of Physicians.

in the conducting airway must be subject to aerodynamic variables which are independent of the original separation of dead space gas from alveolar gas.

However it has been found possible, by simultaneous measurement of nitrogen concentration and volume flow of expired gas, to separate dead space gas from alveolar gas by an application of Bohr's formula. Throughout this paper, the term 'physiological dead space' will be used to refer to the volume of the conducting airway down to the location at which a large change in gas composition occurs. This measurement of physiological dead space will change not only with alterations of the caliber of the airway, but also with the extent of boundary diffusion between the alveolar and dead space gases; the latter, among other factors, is a function of time and hence of the rate of respiration.

The effective ventilation of the residual air by a given tidal volume is reduced by the physiological dead space and also by non-uniform ventilation of the residual air. Therefore ineffective tidal volume has these two components; it is believed that the method to be described may differentiate the two.

#### METHODS

This method is based upon the continuous and simultaneous measurement of *a*) expired gas volume-flow and *b*) expired gas  $N_2$  content, following the change from breathing air to breathing 99.6 per cent oxygen.

1.  *$N_2$  Analysis.* The nitrogen meter developed by Lilly and Hervey (8) continuously samples (using about 1.0 cc/sec.), analyzes and records the nitrogen content of constant composition samples with an accuracy from day to day of  $\pm 2$  per cent  $N_2$ , relative to Haldane analyses. This is an overall accuracy summing errors in sampling methods, measurement of records and setting the instrument. In our instrument the response to a step-wise change in gas composition occurs with a delay of 0.03 to 0.05 second between the time the sample enters the instrument and the time at which the record starts to change; 95 per cent of the final response is attained within an additional 0.03 second and maximal response is attained 0.10 to 0.12 second after the sample enters the instrument. The delay in the final 5% of response may not be instrumental, but due to our inability to produce a completely square gas front.

2. *Volume-flow Analysis.* Expired gas is passed through a flow meter, which continuously measures the pressure differential across a 400-mesh screen in the flow path, by means of an electrical capacitance manometer (9). Photographic recording of  $N_2$  concentration and flow is made by appropriate oscillograph galvanometer-camera systems. With the flow meter connected as described below, the response to a step-wise change in flow occurs with a starting delay in recording of not more than 0.03 second and maximal response is attained in an additional 0.05 to 0.07 second. The flow meter response is calibrated daily by blowing compressed air through a rotameter to it. Volumes presented hereafter are derived without temperature correction from the rotameter calibrated at 760 mm. Hg and 21.1°C. The area of the flow tracing, as it is deflected from the zero line by an expired breath, can be converted to a volume measurement by measurement with a planimeter or with a squared transparent sheet. The error in volume measurements by this method is

$\pm 5$  per cent; this combines errors in rotameter ( $\pm 2\%$ ), flow meter calibration and record measurement. In tables 1, 2, 3, 4 and 6, the figures for tidal volume represent the average of three successive expirations. Since the various patterns of breathing were maintained for at least 8 to 10 breaths, it is reasonable to assume that the expiratory volumes were not greatly different from inspiratory or tidal volume.

TABLE 1. PHYSICAL AND RESPIRATORY DATA ON 45 MALE AND 4 FEMALE SUBJECTS

	AGE	HEIGHT	WEIGHT	EXP. VOL. TO WASH OUT D.S. <sup>1</sup>	PHYSIOL. DEAD SPACE <sup>1</sup>	TIDAL VOL. <sup>1</sup>	P.D.S. T.V. $\times 100$	RESP/MIN.
<i>45 Male Subjects</i>								
	yr.	in.	lb.	cc.	cc.	cc.		
Mean.....	26.4	70.8	161.5	325	156	651	25.9	15.6
S.E. mean ( $\pm$ ).....	0.9	0.4	3.3	10	4	33	1.1	0.6
S.D. ( $\pm$ ).....	5.7	2.5	22.3	65	28	222	7.6	4.1
Coeff. variation.....	22%	3.5%	14%	20%	18%	34%	29%	26%
Range.....	19-38	65-77	120-210	207-472	106-219	276-1448	10-43	8.5-27
<i>4 Female Subjects</i>								
Mean.....	24	63.5	120	250	104	589	20	15.5

<sup>1</sup> Average, first three breaths.

TABLE 2. EFFECT OF VOLUNTARY HYPERVENTILATION ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	QUIET BREATHING		VOLUNTARY HYPERVENTILATION	
	Tidal vol.	Physiol. D. S.	Tidal vol.	Physiol. D. S.
	cc.	cc.	cc.	cc.
20	636	111	3085	167
21	478	157	2500	203
22	476	202	1410	253
23	628	147	2105	242
24	683	179	1540	261

TABLE 3. PHYSIOLOGICAL DEAD SPACE IN VOLUNTARY AND EXERCISE HYPERPNEA

	SUBJ. NO. 34 (MALE)			SUBJ. NO. 48 (FEMALE)		
	Tidal vol.	Physiol. D.S.	Insp. time	Tidal vol.	Physiol. D.S.	Insp. Time
	cc.	cc.	sec.	cc.	cc.	sec.
Voluntary hyperventilation.....	910	186	1.1	1200	170	0.9
Post-exercise hyperpnea.....	810	188	1.0	1310	158	1.3
Quiet breathing.....	580	168	1.7	895	105	2.5

3. *Other Apparatus.* A nose clip and rubber mouthpiece of 2 cm. internal diameter were used. The mouthpiece was connected to a four-way metal valve of 2.1 cm. internal diameter. Instrumental dead spaces were respectively 40 cc. and 60 cc. when room air or oxygen was breathed. Oxygen ( $99.6\% \pm 0.1\%$ ) was delivered from a high pressure tank through a demand valve designed to operate with



TABLE 4. EFFECT OF END-INSPIRATORY LUNG VOLUME ON PHYSIOLOGICAL DEAD SPACE

START OF INSPIRATION AT	SUBJ. NO. 34 (MALE)			SUBJ. NO. 48 (FEMALE)		
	Tidal vol.	Physiol. D.S.	Insp. time	Tidal vol.	Physiol. D.S.	Insp. time
	cc.	cc.	sec.	cc.	cc.	sec.
Max. exp. pos.....	440	119	1.3	660	84	2.2
Normal exp. pos.....	580	168	1.7	895	105	2.5
High insp. pos.....	650	233	1.2	830	202	1.1

TABLE 5. EFFECT OF BREATHHOLDING ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	QUIET BREATHING			BREATHHOLDING			
	Exp. No.	Insp. time	Physiol. D.S.	Exp. no.	Insp. time	Physiol. D.S.	Diff. of mean D.S.
		sec.	cc.		sec.	cc.	
50	1	2.0	132	2	20	70	62
	3	2.0	128	4	21	66	
51	1	1.3	160	2	20	95	82
	3	1.3	173	4	21	74	
34	1	2.0	168	2	22	107	61
48	1	2.5	107	2	21	56	52
	3	2.2	114	4	20	61	
52	1	2.5	192	2	22	136	44
	3	2.1	178	4	21	145	

TABLE 6. EFFECT OF SMALL INCREASE OF INSPIRATORY TIME ON PHYSIOLOGICAL DEAD SPACE

SUBJECT	TYPE OF RESPIRATION	PHYSIOL. D. S.	TIDAL VOL.	INSP. TIME	PHYSIOL. D.S. a-b	TIME b-a
		cc.	cc.	cc.		
53	a. Fast insp. & exp.	218	1350	1.3	83	3.9
	b. Slow insp. & exp.	135	1350	5.2		
54	a. Fast insp. & exp.	228	770	1.0	50	1.5
	b. Slow insp. & exp.	178	1060	2.5		
55	a. Fast insp. & exp.	229	1130	1.4	70	1.5
	b. Slow insp. & exp.	159	1160	2.9		
21	a. Regular rhythm	172	572	1.1	61	2.7
	b. Short insp. pause	116	544	3.8		
48	a. Regular rhythm	100	765	1.9	24	1.9
	b. Short insp. pause	70	742	3.8		

low inspiratory resistance. Expired gas was conducted through the four-way valve, a Saddle valve and 30 inches of flexible rubber tubing with 2 cm. internal diameter to the flow meter.

The sampling needle of the nitrogen meter was inserted into the middle of the mouthpiece lumen just external to the subject's lips, adding an apparatus *expiratory* dead space of 2 to 3 cc. Since measurements of physiological dead space were made during expiration, no apparatus correction was made.

Subjects sat quietly in a chair and breathed room air through the mouthpiece for several minutes. The oxygen system was flushed and, during an expiration, the room air orifice was closed so that oxygen was breathed on the following inspiration and thereafter.

4. *Subjects.* The subjects were healthy white males and females between the ages of 19 and 38 years. Certain physical characteristics are given in table 1.

5. *Analysis of Records.* Figure 1 shows the type of record obtained when oxygen is breathed after breathing room air. During inspiration, oxygen (0.4% N<sub>2</sub>) is inhaled. The expired gas may be divided into three nitrogen-fraction phases (8): the first part, approximately 20 to 100 cc. of oxygen and water vapor, represents inspired gas remaining in the upper respiratory tract; a final portion with relatively constant N<sub>2</sub> content probably represents 'alveolar' gas; a mid-portion of about 100 to 300 cc. of gas with a rapidly rising N<sub>2</sub> content represents a mixture of pure inspired gas and alveolar gas, the mixing presumably being accomplished by boundary diffusion and by expiratory flow conditions in the upper airway.

From the nitrogen and flow curves one can obtain the data to solve Bohr's formula for dead space:

$$V_e \times C_e = (V_e - V_{ds}) C_a + V_{ds} \times C_i \text{ in which}$$

$V_e$  = Volume of expired air.

$C_e$  = Concentration of a gas in  $V_e$ .

$V_{ds}$  = Volume of the dead space.

$C_a$  = Concentration of the same gas in alveolar air.

$C_i$  = Concentration of the same gas in inspired air.

This states that an expired breath consists of a mixture of two parts, each with a definite concentration of a given gas.  $V_e$ ,  $C_i$  (approximately zero for N<sub>2</sub>) and  $C_a$  (the N<sub>2</sub> concentration of the alveolar phase) may be measured directly from the record.  $C_e$  is obtained by correcting the N<sub>2</sub> curve for flow variations and measuring the area under it, thus obtaining volume of N<sub>2</sub> expired; this is divided by total volume expired,  $V_e$ , to give  $C_e$ . The equation may then be solved for dead space. Measurements made in this way will be called 'calculated' physiological dead space.

The factor of non-uniformity of alveolar gas is eliminated as follows: The O<sub>2</sub> remaining in the conducting airway after inspiration is mixed in the airway on expiration with alveolar gas similar to that expired immediately after the dead space has been washed out. The N<sub>2</sub> concentration of this alveolar gas is measured and inserted in Bohr's formula. Similarly  $V_e$  is taken as the volume expired up to the point at which this initial alveolar concentration is reached. If the N<sub>2</sub> content of alveolar gas is uniform, i.e. the alveolar plateau is flat, the same result is obtained if

any volume concentration point is selected after the dead space has been washed out. If the  $N_2$  content of alveolar gas is not uniform, the use of a volume-concentration point early in the alveolar plateau largely eliminates the effects of uneven alveolar  $N_2$  content. This point is selected by drawing a straight line along the top of the alveolar plateau and extending it to the left. The point at which the rising  $N_2$  curve first touches this line is taken for measurement of the alveolar concentration.<sup>4</sup>

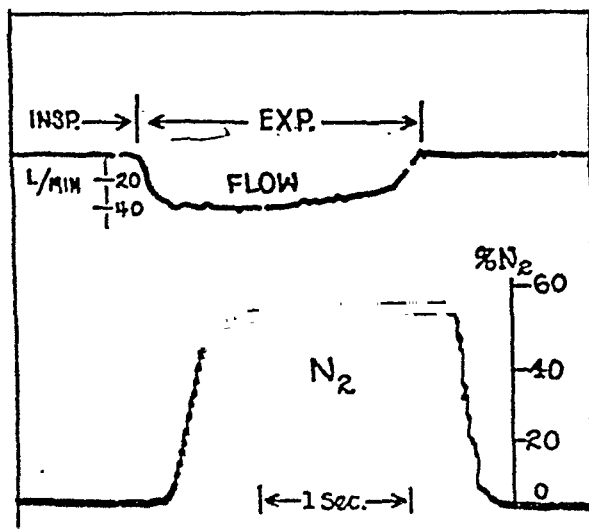


Fig. 1. RECORD OF FLOW and  $N_2$  concentration of expired gas after  $O_2$  inhalation.

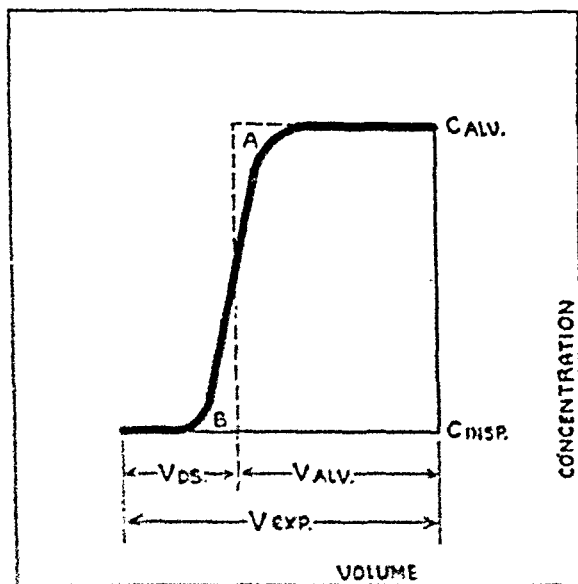


Fig. 2. GEOMETRIC REPRESENTATION of Bohr's formula applied to an expiration (see text for explanation).

Since the preceding calculation is laborious, it was attempted to see how accurately physiological dead space could be estimated directly from the photographic record. Assuming a constant expiratory flow, an expiration may be represented geometrically by figure 2. The area under the curve equals  $V_e \times C_a$ , which by the

<sup>4</sup> Records of normal subjects show, in practically all cases, an approximately rectilinear plateau; in some subjects with pulmonary disease the plateau is curved and the initial alveolar point cannot be reported in this way.

Bohr equation also equals the sum of the two products,  $V_{ds} \times C_i$  and  $C_a \times (V_e - V_{ds})$ . Since  $C_i$  lies on the abscissa,  $V_{ds} \times C_i$  contributes no area. Therefore the area under the curve equals  $C_a \times (V_e - V_{ds})$ .  $C_a$  is known, and  $V_e - V_{ds}$  may be found by constructing a rectangle which has  $C_a$  as one side and which has an area equal to that area under the curve. This is done by dropping a perpendicular to the abscissa such that area A equals area B.

In applying this procedure to the photographic record, a perpendicular is drawn on the nitrogen meter record such that area A equals area B, as in figure 2. This is estimated visually with the help of a squared transparent ruler. The perpendicular is extended to cross the flow curve. The area of the flow curve to the left of the perpendicular is measured, converted to cc. and is called the 'estimated' physiological dead space.

#### POTENTIAL SOURCES OF ERROR IN METHODS

1. *Instrumental Delay.* Figure 3 shows an expiratory record (*above*) and a record obtained with a stepwise change in flow and  $N_2$  content (*below*). With quiet

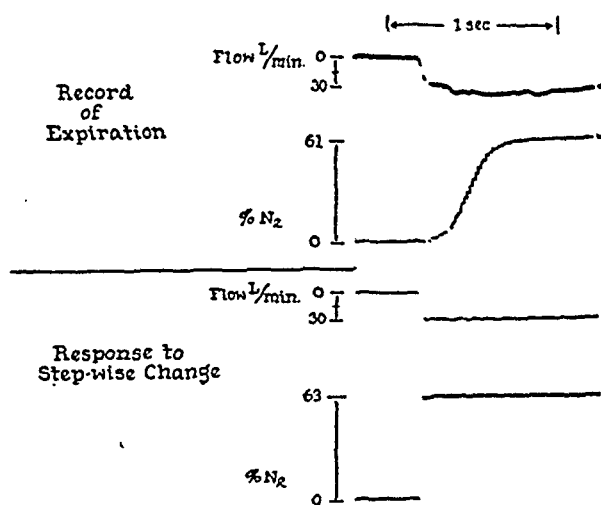


Fig. 3. COMPARISON OF INSTRUMENTAL LAG and changes recorded during expiration (see text for details).

or rapid expiration, the change of  $N_2$  concentration occurs over a period considerably longer (4- to 8-fold and 2- to 4-fold respectively) than the instrumental delay. Likewise the sustained high level of expiratory flow rates is attained only after a delay several times longer than the instrumental lag. Therefore the analyses of  $N_2$  and flow should be reasonable representations of the actual events.

2. The 'estimation' method assumes arithmetic scales on the ordinate and abscissa of figure 2. The non-arithmetic scale of the nitrogen meter response (fig. 1) should make the estimations about 5 per cent too large. Flow variations which occur during the period of rapid  $N_2$  change may introduce error. Actually those flow variations are small because *a*) more than 75 per cent of peak flow is attained early in expiration, before  $N_2$  begins to rise rapidly, and *b*) the apparatus has a damping effect. The 'calculation' method is not subject to these errors. Therefore comparison was made between the values obtained by 'calculation' and by 'estimation' in 14 different breaths including quiet and rapid expirations performed by 6 subjects. Estimated values varied both above and below the calculated values. The mean difference between estimated and calculated was 3.4 cc. and the standard error of the mean

difference was  $\pm 2.6$  cc., indicating that there was no significant difference between values obtained by the two methods.

Further checks on the 'estimation' method were made as follows: *a*) artificial dead spaces were made by connecting rubber hoses to the apparatus mouthpiece, filling the hoses with oxygen and then flushing them through the apparatus with air or gas mixtures containing 63 per cent  $N_2$ -37 per cent  $O_2$ , or 58 per cent  $N_2$ -42 per cent  $O_2$ , at flow rates of 15 to 35 l/min. The internal volume of the hoses was measured by filling with water. Three estimations of an 85-cc. smooth bore hose, 2.0-cm. internal diameter, were 89, 93 and 82 cc. Four estimations of a 170-cc. corrugated hose, internal diameter 2.0 to 3.0 cm., were 175, 164, 160 and 175 cc. Six estimations of a 205-cc. corrugated hose were 197, 194, 194, 213, 203 and 206 cc. *b*) Physiological dead space of a subject was 'estimated' to be 168 cc. Then an additional 200 cc. of dead space was added by placing a smooth bore hose in the mouth. Estimations were found to be 373 and 358 cc., differences of 205 and 190 cc.

From the evidence above, it appears that the 'estimation' method, which is technically much easier, is as suitable as the 'calculation'. The data presented here-

TABLE 7. EFFECT OF VARYING INSPIRATORY VOLUME-FLOW ON PHYSIOLOGICAL DEAD SPACE<sup>1</sup>

SUBJECT	PRE-INSPIRATORY POSITION	END-INSPIRATORY POSITION	INSPIRATORY VOLUME	INSPIRATORY TIME	MEAN INSPIRATORY FLOW	PHYSIOLOGICAL DEAD SPACE
			cc.	sec.	cc./sec.	cc.
34	Max. exp.	Max. exp. + 1760 cc.	1760	1.7	1040	169
	Normal exp.	Max. exp. + 1730 cc.	600	1.5	400	176
30	Max. exp.	Max. exp. + 1570 cc.	1570	1.2	1300	178
	Normal exp.	Max. exp. + 1870 cc.	625	1.2	520	188

<sup>1</sup> All figures average of three breaths.

after were obtained by 'estimation'. In the absence of any direct or absolute method of measurement of physiological dead space (experiments on gas mixing in tubes are not ideal reproductions of aerodynamic conditions in the airway), it is impossible to estimate the accuracy of the values obtained on subjects. These values are, however, similar to those obtained by authors (6) whose methods have eliminated the effects of non-uniformity of alveolar air.

3. *Subjective Error in Estimation.* In 45 male subjects listed in table 1, estimations of physiological dead space were made by one person on three successive breaths of oxygen, usually the first three breaths. If the physiological and instrumental factors influencing the measurement are assumed to be constant, then the variation between measurements may be said to be due to individual accidental error in the estimation. Although the alveolar  $N_2$  decreases with successive breaths, there was no consistent variation of estimated dead space values between the first and later breaths. The standard deviation of the differences between the individual estimations and the mean of the three was  $\pm 8.9$  cc. The figures in table 1 represent the mean of the three estimations. In the other experiments where comparisons of change in one individual are made (tables 2-4, 6) single figures represent the mean of

measurements on three successive similar breaths. The standard error of these means is  $\pm 5.1$  cc., the standard error of the difference between two means is  $\pm 7.2$  cc. and a difference of 20 cc. or more is significant.

## RESULTS

1. The volume of gas required to wash out the dead space on expiration, after which 'alveolar' gas is expired, is shown in table 1 to have an average value of 325 cc. Figure 2 shows that this volume, tentatively called 'kinetic dead space' by Lilly (8), is larger than that of the physiological dead space, since it includes both the pure inspired gas remaining in the airway after inspiration and also some alveolar gas, which is mixed in the airway with inspired gas during expiration.

2. Some alveolar gas appeared in the expired air, as shown by the start of a rising  $N_2$  content, after an average of 41 cc. expired by subjects in table 1. The accuracy of this measurement is limited by the flow meter lag and by the relative insensitivity of this  $N_2$  meter at very low  $N_2$  fractions.

3. Table 1 shows the volume of the physiological dead space found in 45 normal men, sitting and breathing naturally. The average is 156 cc. with an 18 per cent coefficient of variation. Measurements in 4 women gave smaller values, averaging 104 cc.

4. Radiologic and bronchoscopic evidence has shown that the bronchial tree increases in volume with inspiration. Thus, anatomical changes could affect the volume of the physiological dead space. Also the demarcation of gas concentrations between the terminal bronchioles and the alveolar spaces must be affected by diffusion. The effects of these two factors were shown in the following experiments.

*A. Anatomical effects.* Measurements made on 5 men, during quiet breathing and during voluntary hyperventilation, are shown in table 2. Large increases in tidal volume resulted in increases of 46 to 95 cc. in physiological dead space. A comparison of voluntary hyperventilation and hyperpnea due to exercise (one minute after knee bends) was made in 2 subjects. Table 3 shows that there was no significant difference between the increased physiological dead space found with the two types of hyperpnea. In view of Verzar's data on changes in total lung volume during and after exercise (20), it is possible that different results would have been obtained on measurements during exercise, which were precluded by apparatus immobility. The significance of the notation of inspiratory time in table 3 will be discussed below.

The increased physiological dead space found with larger tidal volumes is presumably due to the increase in anatomical volume on deeper inspiration. Further demonstration was obtained by experiments on 2 subjects in whom oxygen inhalation was started at *a*) maximal expiratory position, *b*) normal quiet expiratory position and *c*) almost maximal inspiratory position. The maximal differences in end-inspiratory lung volumes were 3000 to 3500 cc.; tidal volume and rate were voluntarily made similar at all three lung positions. Table 4 shows that at reduced lung volumes the physiological dead space was definitely decreased. With an increase of about 3000 cc. in lung volume, the physiological dead space increased about 100 cc.

*B. Diffusion effects.* Experiments were made on 5 subjects in which the time available for diffusion of  $N_2$  between terminal bronchioles and the more peripheral spaces was prolonged by holding the breath for about 20 seconds in the normal inspiratory position. Table 5 shows a definite decrease in physiological dead space with breathholding in every case, presumably because the peripheral boundary of pure inspired gas had receded up the bronchial tree.

In 3 male subjects, diffusion time was varied within physiological limits by voluntarily breathing with *a*) rapid inspiration and expiration and *b*) slow inspiration and expiration. In 2 subjects,

breaths with a short end-inspiratory pause were alternated with uninterrupted breaths. Table 6 shows that the physiological dead spaces were smaller with the slow rate and that 2- to 3-second inspiratory pauses also resulted in a decrease.

5. To test the proposal (5) that physiological dead space is decreased by higher rates of inspiratory flow, experiments were done on 2 subjects in which inspiratory time and end-inspiratory lung volume were constant, but the rate of inspiratory volume flow was varied. This was accomplished by analyzing breaths which ended at similar inspiratory positions but started at varying expiratory levels. Inspired volumes were measured on a 6 L. recording spirometer used for the source of  $O_2$ . Table 7 shows that increasing the mean inspiratory volume flow by about 2.5 times did not significantly affect the volume of physiological dead space.

#### DISCUSSION

Since the physiological dead space may change in any one individual with respiratory rate and depth, a 'normal' value for a group with varying rates and depths of respiration, and varying anatomy, is not very meaningful. However the average value of 156 cc. for resting males agrees closely with the commonly accepted value of 150 cc. and the 104-cc. average for 4 females is similar to Lindhard's average value of 92 cc. for 5 females (10). On the other hand, Kaltrieder *et al.* (11) found an average value of 256 cc. in a group of 50 males, 38 to 63 years old; if the non-uniformity of alveolar ventilation is increased in older 'normal' males (1), a larger value would be expected when calculations are based on Haldane-Priestly alveolar air samples. The method described in this paper has the advantages of knowing the concentration of alveolar gas which immediately follows that alveolar gas which washes out the dead space and of not requiring the cooperation of the subject in obtaining a sample of alveolar gas. It is probable that these factors are partly responsible for the smaller coefficient of variation in this group (18%) as compared to that in Kaltrieder's series (43.5%).

A recent text (12) illustrates the calculation of alveolar ventilation with *a*) a dead space of constant volume and *b*) a constant dead space/tidal volume fraction. Our data show that dead space volume is not constant; also it is a variable fraction both of tidal volume in different individuals and of different tidal volumes in one person.

Many authors have found that physiological dead space increases with increasing lung volume. However, in our experiments, the increase from maximal expiratory position to maximal inspiratory position was only of the order of 100 to 150 cc., as Krogh (3) and others (6, 13) have also found. Combining the anatomical data of Rohrer (14) and the radiological data of Huizinga (15), one can calculate that the volume of the airway, from the glottis to the intralobular bronchioles of the fifth order, would increase by about 230 cc. from deep expiration to deep inspiration. The smaller increase measured by physiological methods may perhaps be explained by the time that elapses between the first exposure, during early inspiration of inspired gas in the bronchioles, to the gas of alveolar ducts and alveoli and the exit and analysis of this gas on the succeeding expiration. During this time, diffusion exchange will occur between the bronchioles and the alveoli and the dead space measured by physiological methods will be smaller than the anatomical volume.

Henderson *et al.* (16) and Haldane (17) found that the dead space for  $\text{CO}_2$  and  $\text{O}_2$  decreased if the breath was held after inspiration; Grosse Brockhoff and Schoedel (5) were unable to confirm this. Krogh and Lindhard (3) and Mundt (6), using hydrogen methods, noted a decrease but it is doubtful if results obtained with such a rapidly diffusing gas should be applied to the normal respiratory gases. Our data show that prolongation of inspiratory time by only two seconds, such as may result from a prolonged inspiration, will decrease significantly the physiological dead space. Thus with deep slow respiration, the anatomical increase will be counteracted by the prolonged time available for diffusion between the terminal bronchioles and the alveoli. The finding that a 20-second prolongation of inspiratory time (table 5) does not reduce the physiological dead space a great deal more than a two- to four-second prolongation (table 6) is not surprising. In the respiratory bronchioles, for example, the diffusion course is short and only a few seconds or less are required to greatly reduce initial concentration differences (19). The effect of diffusion in the larger bronchioles will be less evident because their total volume becomes progressively smaller toward the large bronchi; also as diffusion courses become longer, time must be prolonged exponentially to accomplish similar degrees of concentration equalization.

Our finding that physiological dead space is unaffected by increased inspiratory volume flow does not support Grosse-Brockhoff and Schoedel's proposal that bronchiolar-alveolar mixing is facilitated by turbulence resulting from faster inspiratory flow, with a resulting reduction of dead space volume. While it cannot be said that turbulent flow and attendant mixing do not occur, diffusion alone should accomplish mixing between the main stream and the alveoli along the respiratory bronchioles and alveolar ducts because the distances involved are so small in relation to normal respiratory times.

The measurements of respiratory dead space by the method described may have several clinical uses: *a*) patients with pulmonary abnormalities may have large ineffective tidal volumes (2, 18); this method may be able to demonstrate whether an enlarged physiological dead space or uneven intrapulmonary gas mixing is responsible. *b*) It may be possible to employ the method to measure physiological or pharmacologically induced changes in bronchiolar caliber.

#### SUMMARY

1. Physiological dead space was measured by simultaneous and continuous measurement of volume flow and  $\text{N}_2$  content of gas expired following the change from breathing air to breathing 99.6 per cent  $\text{O}_2$ . In normal subjects the effect of non-uniform alveolar gas on dead space measurements can be largely eliminated.

2. The average volume of the physiological dead space in 45 healthy males at rest was 156 cc.; the average expired volume required to wash out the dead space was 325 cc. The physiological dead space/tidal volume fraction averaged 25.9 per cent, but varied widely in different individuals.

3. The volume of the physiological dead space is affected by: *a*) anatomical volume of the bronchial tree. Maximal variations of inspiratory lung volume changed dead space by about 100 cc. Voluntary hyperventilation and post-exercise hyperpnea increased physiological dead space equally; the increase was 100 cc. or



less. b) Gas diffusion between terminal bronchioles and alveolar spaces. Prolongation of inspiratory time by two to three seconds significantly reduced the volume of the physiological dead space and breathholding, during inspiration (20 sec.), caused reductions of 44 to 82 cc. In slow deep breathing the anatomical volume increase is counteracted by diffusion occurring during the prolonged inspiratory time.

4. Variations in the rate of inspiratory volume flow did not affect the volume of the physiological dead space.

5. Clinical applications of the method are suggested.

The author wishes to express his thanks to Dr. J. H. Comroe, Jr. for his advice and encouragement and to Adele Kynette and Nancy Krafft for technical assistance.

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# RÔLE OF THE VAGI IN THE CROSSED PHRENIC PHENOMENON

PAUL O. CHATFIELD AND SEDGWICK MEAD<sup>1</sup>

*From the Department of Physiology, Harvard Medical School*

BOSTON, MASSACHUSETTS

THE term 'crossed phrenic phenomenon' refers to the recovery of activity in a hemidiaphragm paralyzed by hemisection of the spinal cord above C<sub>3</sub>, when the contralateral phrenic nerve is cut or blocked in various ways.

This phenomenon was observed by several earlier workers (1-3) and it was found (3) that the crossing of descending respiratory impulses occurred at the level of the phrenic nuclei.

Deason and Robb (4) showed that crossing could be produced (in dogs and cats) under several conditions, even without previous phrenic section. Thus they found crossing to occur with 'dyspnoea' (not further described), traction on the active phrenic not great enough to stop conduction and on stimulation of the sciatic.

Rosenblueth and Ortiz (5) found that crossing occurred—on section or block of the active phrenic—in dogs, cats, rabbits and woodchucks, but not in monkeys or guinea pigs. In dogs only, crossing also occurred regularly on section of the vago-sympathetic trunks. They concluded that the crossing was not due to asphyxia or to interruption of inhibitory afferent impulses in the phrenic or vago-sympathetic nerves.

After a subsequent study Rosenblueth, Klopp and Simeone (6) were forced to conclude that crossing is due to block of phrenic motor impulses and that the central changes—at the level of the phrenic nuclei—which permit crossing are mediated by some process not involving the conduction of nerve impulses.

Tosatti (7) found that the phrenic nucleus has a double innervation from the respiratory center—a principal homolateral tract running in the lateral column of the cervical cord and a reserve tract running in the anterior column and composed of both direct and crossed fibers. He held that crossing was due to activation of this crossed pathway, perhaps by elevation of the blood CO<sub>2</sub> concentration, which he showed occurred after inactivation of the functioning phrenic.

Finally Seligman and Davis (8) found that crossing was produced by prostigmin, eserine, acetylcholine (protected by prostigmin) and strychnine. They also showed that crossing occurred with asphyxia and after section of the vagi if prostigmin had been given previously.

Thus in several species crossing can occur, under various conditions, without interruption of the remaining phrenic motor activity. The present study is a further investigation of the crossed phrenic phenomenon with emphasis on the rôle of the vagi.

## METHOD

Our conclusions are based on observations made on 23 rabbits. At first dial (Ciba, 0.5 cc/kg.) and later nembutal (Abbott, 0.4-0.6 cc/kg.) were used as anesthetics intravenously. The kind of anesthetic used did not appear to affect the results, although the rabbits tolerated nembutal better than dial.

Diaphragmatic contractions were at first recorded from Head's slips (anteriorly

Received for publication April 12, 1948.

<sup>1</sup> Research Fellow in Medicine, Harvard Medical School.

placed bands of diaphragmatic fibers, well developed in the rabbit) with isotonic levers—in such records inspiratory excursions are upward. Later records were made directly from the domes of the diaphragm after the abdomen had been widely opened. In these records the inspiratory excursions are downward. The use of Head's slips was abandoned when it became apparent that in many animals the slips did not contract even when the diaphragm was contracting well. We came to regard Head's slips as accessory muscles of respiration, since contraction could often be induced in quiescent slips by having the animal breathe against resistance.

The respiratory effects of all procedures used were checked by direct visual observation of the diaphragm.

In all animals a tracheal cannula was inserted and the cord hemisected between C<sub>2</sub> and C<sub>3</sub> to produce a respiratory hemiplegia. The vagi and phrenics were approached in the neck. The phrenics were inactivated by section or by block with ether or ice. Stimulation was produced through shielded electrodes using a Grass model 3 monophasic square-wave stimulator with variable pulse duration, frequency and intensity.

## RESULTS

*A. Rôle of Hering-Breuer Afferents.* As has been mentioned, in some animals with a cervical hemisection Head's slips did not contract with respiration even on the unparalyzed side. In such circumstances, partial occlusion of the tracheal cannula led to contraction of the slips both on the unparalyzed and occasionally on the hemiplegic side. As can be seen in figure 1, the contractions started with the very next breath after partial occlusion, a characteristic of Hering-Breuer afferent activity.

Even when the normal Head's slip was contracting, partial occlusion of the tracheal cannula increased activity on the functioning side and sometimes induced activity on the hemiplegic side.

The crossing produced by partial occlusion could also be shown on records from the domes of the diaphragm (fig. 2A). That this increase in activity was due in part at least to impulses in the vagi was shown by the different character of the response to occlusion after the vagi had been sectioned bilaterally (fig. 2B). In these circumstances respiration did not slow or increase as greatly as when the vagi were intact. Possibly the increased depth and crossing of respiration on occlusion after vagal section was due mostly to asphyxia, since with vagi cut the increase was more gradual, did not become maximal until the animal was visibly cyanotic and persisted longer after the airway had been reopened.

Crossing produced by partial occlusion of the tracheal cannula may be related to the crossing described by Deason and Robb (4) which occurred when their animals were in a state of 'dyspnoea'.

*B. Effects of Section of the Vagi.* As noted above, Rosenblueth and Ortiz (5) found that crossing was readily produced by severing the vago-sympathetic trunks in dogs, but not in monkeys, cats, rabbits, guinea pigs or woodchucks. Rosenblueth, Klapp and Simone (6) showed that sectioning the vagi in cats and rabbits would produce transient crossing if crossing had been produced previously by one or more reversible blocks of the active phrenic. Seligman and Davis (8) produced permanent

crossing on vagal section in one cat and in rabbits which had received prostigmin previously.

In the present experiments, section of the vagi produced transient crossing in 4 out of 8 animals in which it was performed as the initial experimental procedure (fig. 3). The rôle which the vagi may play in the production of crossed respiration is indicated by the fact that in the 4 animals which did not show crossing on vagal section, no crossing occurred on subsequent phrenic block. In addition there was a correlation between this lack of crossing and an inactivity of Hering-Breuer reflexes as indicated by the responses to partial occlusion of the tracheal cannula and to compression of the thorax from without. Normally partial occlusion slowed respiration while compression of the thorax accelerated it. But in a typical animal from

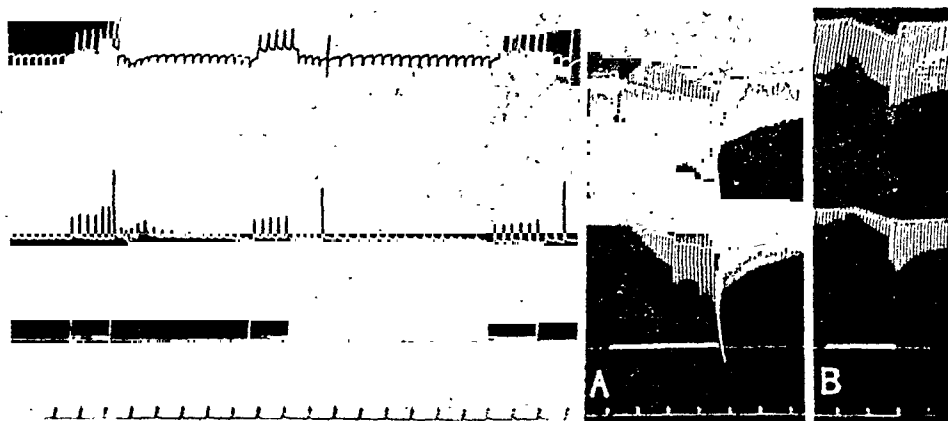


Fig. 1 (left). RECORD MADE FROM HEAD'S SLIPS. The upper record is from the normal side; the lower, from the hemiplegic side. This and all subsequent records are from rabbits given a respiratory hemiplegia by cervical hemisection above C<sub>3</sub>. In this case, Head's slips were not functioning. At signals, partial occlusion of the tracheal cannula. Note that this procedure activated both the normal and hemiplegic slips. Activation occurred with the very next breath after partial occlusion of the cannula. Time interval (bottom signal) in this and all subsequent figures is 10 seconds.

Fig. 2 (right). RECORDS FROM THE DOMES OF THE DIAPHRAGM. Upper record, normal side; lower record, from hemiplegic hemidiaphragm. A. Vagi were intact. At signal, partial occlusion of the tracheal cannula caused crossing which persisted somewhat when the airway was reopened. B. Same preparation as in A, but vagi have been cut, producing slight crossed activity in the previously hemiplegic hemidiaphragm. At signal, partial occlusion of the tracheal cannula. Note difference in the response of the hemidiaphragms from that of fig. 2A. The respiratory movements did not slow or increase as much as they did with vagi intact.

the 'non-crossing' group mentioned above, Hering-Breuer responses were poor, section of the vagi did not produce crossing and ether block of the phrenic was ineffective in producing crossing both before and after bilateral vagotomy.

C. *Effects of Stimulation of Central End of Cut Vagus.* Since partial occlusion of the tracheal cannula could cause crossing, an effect some of which depended on the integrity of the vagi (see above), an attempt was made to reproduce this phenomenon by appropriate stimulation of the central end of the cut vagus. It is now well known (9) that high frequency vagal stimulation results in a slowing of respiration and an expiratory apnea, while low frequency stimulation causes a quickening of respiration and an inspiratory apnea. Accordingly the central end of the cut vagus was stimulated with monophasic square waves of 0.01 msec. duration at rates of 120 to 300 per second. Such stimulation caused the appearance of, or an increase in

crossed activity, an effect which might persist after stimulation had ceased (fig. 4A). That this increase in crossing was probably not due to anoxia from the apnea initially produced by high frequency stimulation is demonstrated in figure 4B, where an increase in crossed activity is shown even though the animal had been breathing 100 per cent  $O_2$  for several minutes before stimulation of the vagus.

Since crossing may occur on section of the vagi, the possibility of inhibitory afferents in the vagi was investigated. Appropriate low-frequency (5 per sec.) stimulation with square waves as before was found to inhibit crossed as well as normal respiratory diaphragmatic movements.

We conclude then that the vagus contains afferent fibers which both stimulate and inhibit crossed respiration. The appearance of crossing after vagal section in some animals could then be due to removal of this inhibition. It might also be

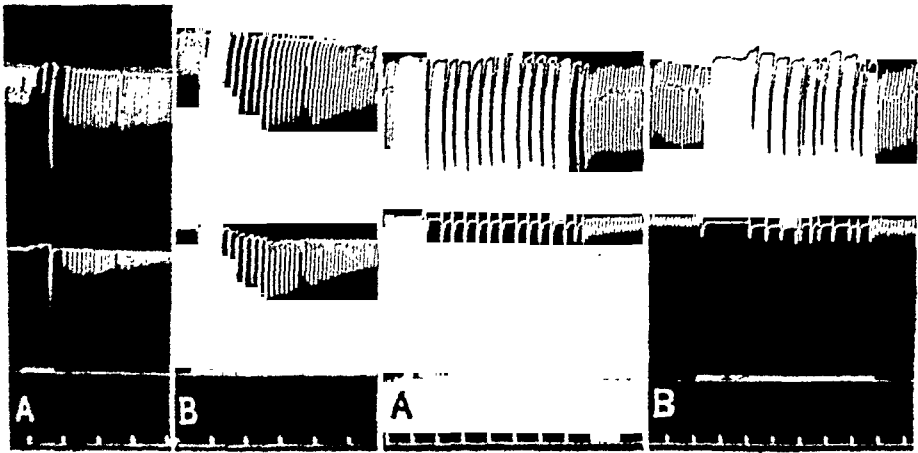


Fig. 3 (*left*). EFFECTS OF SECTIONING VAGI. Upper record from normal hemidiaphragm; lower record from hemiplegic hemidiaphragm. Records from domes. A. At signal, ligation and section of one vagus. Crossing produced. B. Same preparation. At signal, subsequent section of the other vagus. Crossed diaphragmatic contractions again increased.

Fig. 4 (*right*). SHOWING CROSSING PRODUCED BY HIGH FREQUENCY VAGAL STIMULATION (at signal). Lower record is from the hemiplegic hemidiaphragm; upper record from normal side. Records from domes. A. With animal breathing air. Stimulation produced a short period of apnea and increased crossed contractions. B. Same preparation, after animal had been breathing 100 per cent  $O_2$  for several minutes before stimulation. Vagal stimulation still caused crossing.

inferred that crossing after inactivation of the functioning phrenic could likewise be caused by a decrease in vagal inhibition, or by an enhancement of the stimulatory impulses, brought about by some peripheral change.

*D. Relation of the Vagi to Crossing Produced by Block of Active Phrenic.* In spite of the rôle which the vagi may play in the appearance of or inhibition of crossed respiration, block of the active phrenic after crossing had been produced by bilateral vagal section still caused an increase in crossed activity (fig. 5). Asphyxia may play some part in this increased crossed respiratory activity after block of the active phrenic, but it does not furnish the whole explanation. An animal with both vagi cut still showed increased crossing on phrenic block even after it had been breathing 100 per cent  $O_2$  for several minutes previously. Interestingly enough, under these circumstances the crossed activity did not develop to the extent it would have had the animal been breathing air. This result indicates that whatever the mechanism

which brings about the onset of crossed respiration, the crossed pathway—once active—responds as does the normal respiratory pathway to physiological stimuli.<sup>2</sup>

E. *Possible Rôle of Inhibitory Afferents in the Phrenic.* Deason and Robb (4) found that stimulation of the central end of the cut phrenic in cats and dogs increased the rate and amplitude of respiratory movements. They felt that crossing might be initiated by excitatory afferent discharges from the cut end of the nerve.

Rosenblueth and Ortiz (5) stimulated the central end of the cut active phrenic with an inductorium in dogs, cats, rabbits and woodchucks, obtaining variable results. During the present investigation the central end of the cut active phrenic was stimulated with monophasic square-waves whose duration varied from 0.01 to 20 msec., with intensities as high as 100 volts, and with frequencies ranging from 1 to 500 per second. In no instance was any significant effect on respiration noted, though the existence of afferent fibers in the phrenic affecting respiration has been postulated (10-13). On the basis of the available evidence we agree with Rosenblueth and Ortiz that crossing does not result from the removal of inhibitory impulses in the active phrenic.

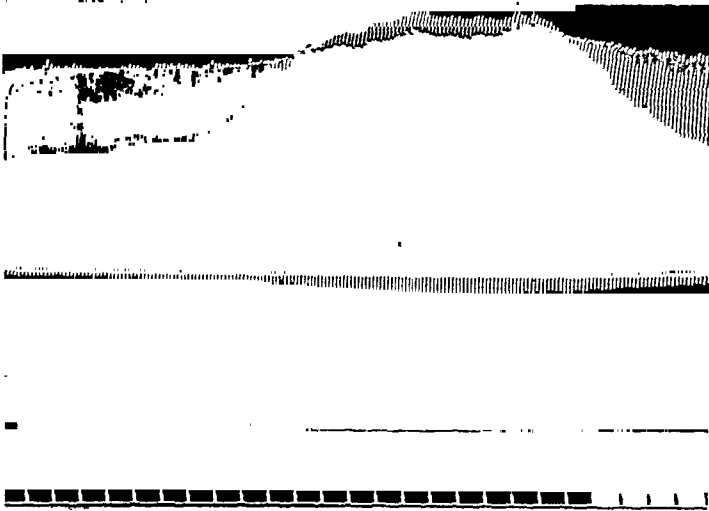


Fig. 5. RECORDS FROM DOMES. Upper, normal side; lower, from hemiplegic hemidiaphragm. Slight crossed activity had resulted from section of both vagi. At signal, ether block of active phrenic. Note further increase in crossed activity after phrenic block.

#### DISCUSSION

It seems obvious that crossing of descending respiratory impulses may occur under various conditions and be brought about by several factors that affect respiration—e.g. asphyxia, drugs and afferent stimulation. From our results it would be tempting to formulate an hypothesis for the crossed phrenic phenomenon based on changes in afferent impulses in the vagi and other nerves brought about by section or block of the active phrenic, with a resulting stimulation of or removal of inhibition from the crossed respiratory pathway. Such an hypothesis could even account for the appearance of, or increase in, crossed respiration on phrenic block after previous section of the vagi, since it has been shown that the lung is supplied with afferents other than those running in the vagi. These afferents have been demonstrated in various species by several investigators (14-17).

Such an hypothesis may account for crossing under certain conditions. How-

<sup>2</sup> We have also been able to activate the crossed pathway directly with the respiratory stimulant nikethamide (Coramine, kindly furnished by Ciba Pharmaceutical Products, Inc., Summit, N. J.). (Unpublished data.)

ever, Rosenblueth, Klopp and Simeone (6) showed conclusively that crossing can occur independent of any change in afferent activity and, under these circumstances, must depend essentially on some change in the phrenic nuclei brought about by severance of the phrenic nerve. The nature of this change remains the most interesting aspect of the crossed phrenic phenomenon. The recent claim of Dolivo and Fleisch (18) that sectioning one phrenic increases the electrical activity of the opposite phrenic, even when all afferent nerves affecting respiration have been sectioned, seems pertinent to a solution of this problem.

#### SUMMARY

The 'crossed phrenic phenomenon' consists of the reactivation of a hemiplegic hemidiaphragm by section or block of the contralateral active phrenic. Experiments on rabbits are reported with reference to the rôle of the vagi in this phenomenon. Crossed respiration was produced by activation of Hering-Breuer afferents by partial occlusion of a tracheal cannula (section A, figs. 1 and 2). Crossing was produced by section of the vagi alone (section B, fig. 3).

Four animals which did not cross on phrenic block or on vagal section showed poor respiratory responses to partial tracheal occlusion and external compression of the thorax (section B). High frequency stimulation of the central end of the cut vagus stimulated crossing (section C, fig. 4). Low frequency stimulation of the vagus inhibited crossing (section C). After vagal section had caused crossing, an increase in crossing still occurred after inactivation of the functioning phrenic (section D, fig. 5). No afferents inhibitory to respiration could be demonstrated in the phrenic nerve with the technic used (section E). The discussion is concerned with the hypothesis that under certain conditions crossing could be produced by changes in afferent impulses from the lungs resulting from inactivation of the phrenic. It is emphasized that the essential problem of the crossed phrenic phenomenon remains to be solved.

We wish to express our appreciation of the assistance rendered during this investigation by Miss Ruth Stern and Mr. David Sheldon.

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# BLOOD SUGAR AND DEXTROSE TOLERANCE DURING ANOXIA IN THE DOG<sup>1</sup>

J. CLIFFORD STICKNEY, DAVID W. NORTHUP AND EDWARD J. VAN LIERE

*From the Department of Physiology, School of Medicine, West Virginia University*

MORGANTOWN, WEST VIRGINIA

**R**ELATIVELY few studies of the blood sugar response of dogs to anoxic anoxia have been reported. McQuarrie *et al.* (1) have determined the hyperglycemic response of fasting dogs exposed for 2.5 hours to 4.5-5.0 per cent O<sub>2</sub>, an extreme degree of anoxia, after a gradual transition from room air. They found increases of between 30 and 150 per cent in the blood glucose level. Lewis *et al.* (2) found no change in blood glucose levels at the end of 24 hours at a pO<sub>2</sub> of 80 mm. Hg. As Kelley and McDonald (3) have recently stated, there are contradictory reports in the literature regarding the effects of anoxia on dextrose tolerance. They found that it was decreased in their 3 dogs at simulated altitudes of 18,000 and 24,000 feet.

We have determined, in strictly unacclimatized dogs, the threshold degree of anoxia which will produce hyperglycemia, the extent of the hyperglycemia at various altitudes and the change in blood glucose level over time at a simulated altitude of 28,000 feet. We have also determined the dextrose tolerance in 7 dogs at a simulated altitude of 28,000 feet.

## METHODS

Ninety healthy normal dogs which, to our knowledge, had never before been exposed to anoxia and which had been fasted approximately 20 hours, were used to study the hyperglycemic response to anoxia. These dogs were exposed without anesthesia to the five simulated altitudes (in a decompression chamber) and for the time intervals shown in table 1. The fasting blood sample was drawn from the saphenous vein immediately before the ascent to the simulated altitude. (Blood samples taken in 20 dogs immediately before and after a 15-minute stay in the decompression chamber at ground level showed that the blood sampling procedure did not elevate the blood sugar in the average dog.) The ascent to the simulated altitude was made rapidly, that is, in about 70 to 160 seconds, depending upon the altitude. The descent at the end of the period of anoxia took 20 to 25 seconds. The second blood sample was drawn within 1.25 minutes on the average after descent.

In 42 of the dogs hemoglobin was determined on the fasting blood sample by the Sahli method.

Seven dogs were used for the studies on dextrose tolerance, but exposure to altitude or the administration of glucose were not repeated more often than once a week in order to minimize the cumulative effects. After taking the fasting blood sample, 1.5 gm. of glucose per kg. of body weight were injected as a 25 per cent solution intravenously. The dog was then exposed immediately to a simulated altitude of 28,000 feet. At 15, 30, 60 and 90 minutes after the beginning of the ascent the dog was returned briefly to ground level (2 min. on the average) for blood sampling. For the dextrose tolerance determination at ground level, the blood samples were drawn at corresponding time intervals. The hyperglycemic response of these dogs to 28,000 feet was determined on blood samples drawn similarly. In all but one dog each procedure was repeated three times.

All blood sugar determinations were made by the method of Folin and Wu.

Received for publication July 12, 1948.

<sup>1</sup> Presented in part before the American Physiological Society in Atlantic City, March, 1948.



## RESULTS

The blood sugar responses for various intervals of time to various degrees of anoxia are given in table 1. It will be seen that 24,000 feet was the lowest simulated altitude at which a statistically significant elevation of blood sugar occurred when the duration of exposure was 15 minutes. The same duration at 28,000 feet produced a much greater elevation and at 32,000 feet the response was still more marked. The average blood sugar level after 15 minutes at the latter was significantly greater than at 28,000 feet ( $p: 0.007$ ).

The hyperglycemic response at 28,000 feet was greatest after an exposure of 30 minutes. As the length of exposure was increased the hyperglycemia at the end of the time interval became less till after 60 minutes the blood sugar level was below that after 15 minutes. It should be emphasized that in order to avoid the use of dogs adapted to anoxia in any degree, the response for each altitude and/or duration of exposure was determined on dogs which had never been used before.

TABLE 1. BLOOD SUGAR RESPONSE OF FASTED DOGS EXPOSED FOR VARIOUS INTERVALS OF TIME TO VARIOUS DEGREES OF ANOXIA

BAROMETRIC PRESS.	SIMULATED ALT.	LENGTH OF EXPOS.	NO. OF DOGS	FASTING BL. SUGAR	FINAL BL. SUGAR	DIFF.	'p'	PER CENT DIFF.
mm. Hg	ft.	min.		mg/100 ml.	mg/100 ml.	mg/100 ml.		
382	18,000	15	10	111	116	5	>0.20	4.5
382	18,000	30	6	102	104	2	>0.20	2.0
328	22,000	15	10	104	108	4	>0.20	3.8
303	24,000	15	10	115	121	6	0.009	5.2
254	28,000	15	14	104	145	41	<0.001	39.4
254	28,000	30	10	110	176	66		60.0
254	28,000	45	7	113	164	51		45.1
254	28,000	60	7	109	131	22		20.2
208	32,000	15	16	110	166	56		50.9

The extent of the elevation of blood sugar produced by anoxia was negatively correlated with the fasting blood hemoglobin concentration, but the degree of correlation was quite low ( $r: -0.42$ ;  $N: 42$ ).

The dextrose tolerance curves at ground level and at 28,000 feet along with the hyperglycemic response of the same dogs are shown in figure 1. The curves are the average results on 7 dogs and are expressed as per cent increases above the fasting blood sugar level which averaged 109 mg/100 ml.

The dextrose tolerance curve during anoxia had a maximum at 15 minutes which was somewhat below that at ground level. In spite of this, at 60 and 90 minutes the curve was elevated and tended to approach the curve of the simple hyperglycemic response to anoxia as the control dextrose tolerance curve approached the fasting level. The average blood sugar levels at 60 and 90 minutes of the dextrose tolerance curve during anoxia were significantly ( $p: 0.01$ ) elevated above the corresponding ones without anoxia. This, of course, indicates that dextrose tolerance was decreased.

A comparison of the hyperglycemic response to simple anoxia (lower curve of fig. 1) when the exposure was interrupted by descents to ground level with that when it was not so interrupted (table 1) reveals that the two were not alike in all aspects. The maximum occurred in the former at the end of 15 minutes while in the latter it occurred at the end of 30 minutes where it was significantly greater ( $p: 0.008$ ). The blood sugar levels at 15 and 60 minutes, however, were about the same for both.

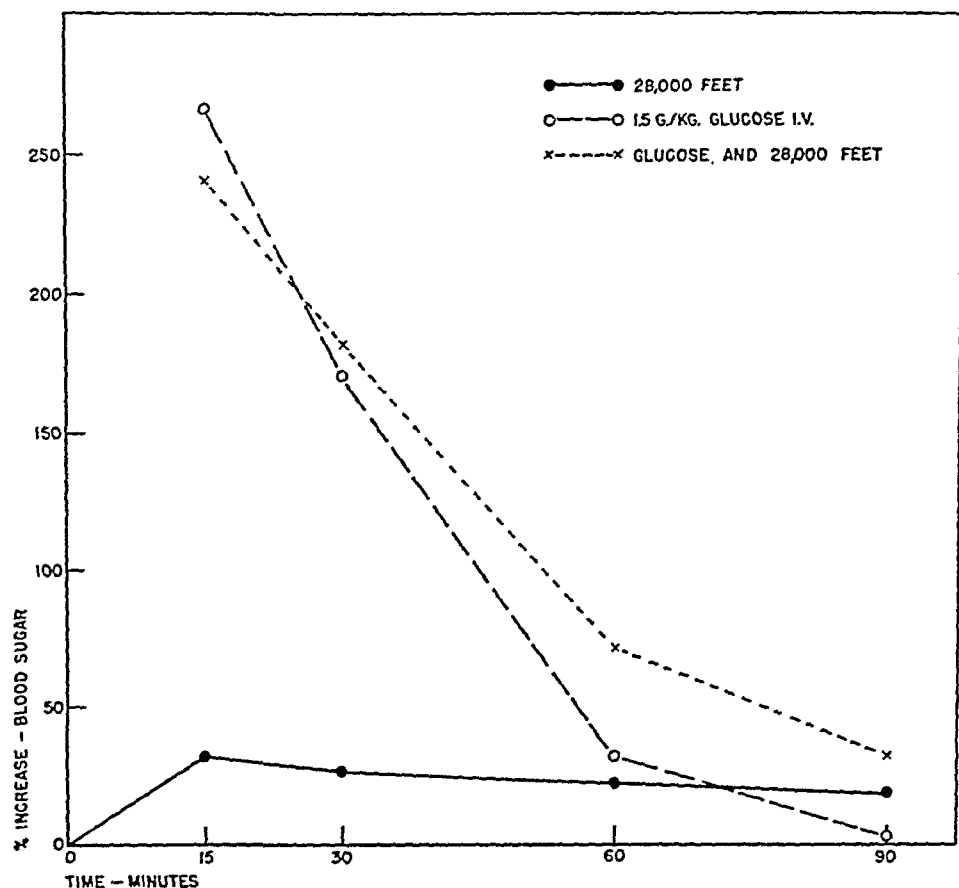


Fig. 1. EFFECT OF ANOXIA on the dextrose tolerance of 7 dogs. Dextrose tolerance at ground level (open circles); dextrose tolerance at 28,000 feet (crosses); hyperglycemic response to interrupted anoxia (filled circles).

#### DISCUSSION

McQuarrie *et al.* (1) have shown that in the dog the adrenal glands are necessary for the hyperglycemic response to anoxia. The blood sugar level reached, however, is due to the participation of both the sympathetic (adrenals) and parasympathetic (pancreatic islets) divisions of the autonomic nervous system in which the former is dominant. The secretion of epinephrine, apparently, is responsible for the blood sugar elevation during relatively short exposures to anoxia.

The work reported here indicates that an altitude of 24,000 feet for 15 minutes can produce a threshold mobilization of blood sugar. Higher altitudes for a similar length of time cause a proportionate increase in the response. The failure of Kelley and McDonald (3) to find an elevation of blood sugar at 24,000 feet can be ascribed

to the short length of exposure (approximately 5 min.), possibly to their failure to use strictly unacclimatized dogs and to the limited number of experimental animals used. The rôle of the sympathico-adrenal system in elevating blood sugar under greater anoxic stress can not be called into question by the results reported by the above authors. The threshold in anoxic stress which will initiate other adaptive reactions of the body is considerably below that for hyperglycemia. The mobilization of blood sugar has long been recognized as an emergency response of the homeostatic mechanisms (4). As might be expected, the duration of exposure is a factor in setting in motion such mechanisms. Thus, the results of our study indicate that a 30-minute uninterrupted exposure to 28,000 feet is as effective as a 15-minute one to 32,000 feet in elevating blood sugar in dogs. Continued exposure at 28,000 feet results in a decline in blood sugar level. Gellhorn and Packer (5), in rabbits, have concluded that prolonged anoxia leads to a loss of the earlier glycogenolytic activity of epinephrine, the secretion of which was presumed to continue. However, the possibility that epinephrine secretion may decline under such circumstances or that other hormonal and/or nervous mechanisms may intervene, have not been strictly excluded.

The percentage increase in blood sugar at the end of 15 minutes in the dextrose tolerance test at altitude was not quite so great as at ground level. The additional mobilization of blood sugar by anoxia might have been expected to cause this point to be elevated above that of the control. This mobilization may not have been present, for Safford and Gellhorn (6) have shown that in the rat the reactivity of the sympathico-adrenal system decreases with rising blood sugar level. It is not apparent (inasmuch as no obvious correlation between fasting blood sugar levels and blood sugar rises due to anoxia could be found in our results) whether or not such an effect holds for the dog. Another possibility is the greater urinary loss of glucose in anoxia. From the work of Toth (7) on unanesthetized dogs it could be predicted that urine excretion would be elevated some 50 to 65 per cent by the degree of anoxia in question here. Since the blood sugar level was above the renal threshold in our experiments, one would have expected a greater loss through glycosuria in the dogs at altitude than at ground level.

The elevated points in the dextrose tolerance curve during anoxia at 60 and 90 minutes are evidence that tolerance was decreased. From inspection of the records, the decreased tolerance appears to be due to the persistence of the hyperglycemic response to anoxia. If the sympathico-adrenal system had initially been suppressed (as suggested by Safford and Gellhorn) by the artificially produced high blood sugar level, it seems to have been activated as the experiment progressed. It is possible that other factors may be involved as suggested by Kelley and McDonald (3): *a*) insufficient oxygen for enzymatic systems involving carbohydrate; *b*) increased adrenal cortical activity. Our results, although obtained under conditions differing in important aspects, confirm those of the latter authors in respect to dextrose tolerance during anoxia in the dog.

#### SUMMARY

The subjection of 90 strictly unacclimatized dogs to simulated altitudes in a decompression chamber has shown that the threshold degree of anoxia for invoking

hyperglycemia in the average dog is that at 24,000 feet for 15 minutes. Exposures to 28,000 and 32,000 feet for the same length of time invoke proportionately greater elevations of blood sugar. When length of exposure at 28,000 feet was varied from 15 to 60 minutes the maximum rise was seen at the end of 30 minutes. Blood sugar level then declined with continued exposure.

Dextrose tolerance at a simulated altitude of 28,000 feet was found to be significantly decreased on the average in the 7 dogs studied.

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# RÔLE OF THE HERRING-BREUR REFLEX UNDER DEEP PENTOTHAL ANESTHESIA

J. C. SCOTT, E. A. REED, D. SARIS AND H. P. REDONDO RAMIREZ

*From the Department of Physiology, The Hahnemann Medical College*

PHILADELPHIA, PENNSYLVANIA

SEVERAL reports have appeared in the literature on the stimulating effect to respiration of pressure on the thoracic wall (1, 2). More recently reports have been published on a reflex response purportedly originating in the thoracic wall of the dog during periods of respiratory arrest induced by intravenous pentothal (3, 4). According to these observers the respiratory response was elicited by light pressure applied to the region of the fourth rib, at a point of common insertion for the scalenus medius and rectus abdominis muscles in the dog. The assumed reflex basis for these results has been questioned (5, 6). The observations to be reported here were undertaken with the hope of quantitatively evaluating the strength of stimulus necessary to elicit this response. Later the experimental procedure was applied to a study of the effects of unilateral and bilateral vagotomy and of unilateral pneumonectomy.

## PROCEDURE

Fifty-six experiments were performed on 40 some dogs. The animals were anesthetized by intraperitoneal injection of 35 mg. nembutal or pentothal sodium per kg. Oxygen was given by intratracheal insufflation at a rate of 0.5 to 3 liters per minute. Intrapulmonic pressure was maintained at atmospheric values. Cyanosis was absent. Respiration was recorded by a tambour connected to an endotracheal tube. The experimental procedure consisted in studying the response to stimulation during periods of respiratory arrest induced by intravenous pentothal or nembutal. In the earlier experiments attempts were made to maintain a constant depth of respiratory depression by controlling the rate of intravenous drip of 2.5 per cent pentothal. In later experiments 1 cc. or 2 cc. of 2.5 per cent pentothal was injected intravenously and periods of arrest varying from a few minutes to over an hour were obtained. In some cases similar results were obtained by using 2.5 per cent nembutal intravenously.

In order to evaluate the effective strength of stimulus, a 1000-gm., 500-gm. or 200-gm. weight was applied to a freely moving piston in contact with the chest wall. The approximate rate of recovery of excitability was estimated by determining the minimal stimulus at 10-second intervals during the period of inhibition. Similar observations were made on 9 dogs subjected to unilateral or bilateral vagotomy and on 6 dogs with unilateral pneumonectomy. In 4 of the latter, care was taken to avoid injury to the scalenus and rectus muscles during the operation. Respiratory rates were also determined on some of these animals under normal unanesthetized conditions for periods up to eight months following the operation.

## RESULTS

Quantitative measurements of the minimal stimulus during periods of arrest were only partially successful. The duration and depth of inhibition varied with the dose

Received for publication July 16, 1942.

and the interval of time between injections. If 1-cc. doses of pentothal were used and a five-minute period of spontaneous breathing between periods of inhibition was allowed, fairly reproducible curves could be obtained. No attempt was made to estimate the concentration in the blood or rate of elimination of the anesthetic. Figure 1 is shown to indicate the type of result commonly obtained with 1-cc. doses of 2.5 per cent pentothal. Nembutal produced longer and apparently shallower periods of inhibition. Reflex response to the weight could be elicited readily in a zone extending from the second to the sixth rib along the sternal margin. Areas of the thoracic wall bordering the spinal column were less sensitive. Section of the scalenus medius and rectus abdominis muscles did not abolish the reflex response during pentothal inhibition. On the other hand, stretching these muscles failed to elicit a response. In two experiments the reflex was obtained by applying light finger pressure to the parietal pleura after resection of the fourth rib and removal of the adjacent thoracic wall. Responses were mainly diaphragmatic and in some instances

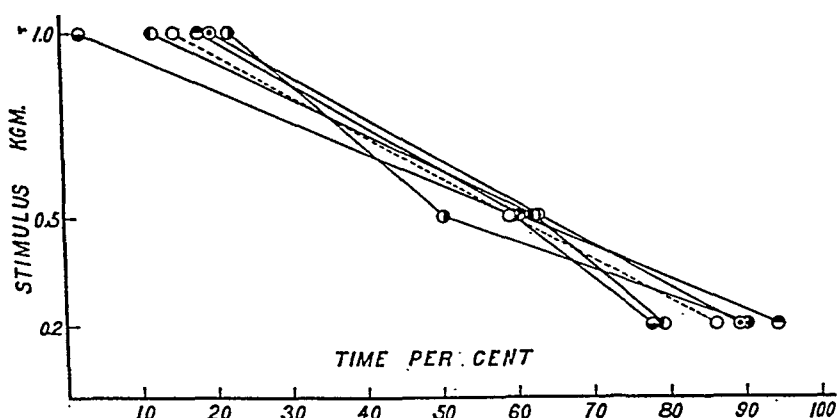


Fig. 1. MINIMAL STIMULUS DURING PERIODS OF PENTOTHAL INHIBITION. The broken line represents the average of a series of runs on the same dog. Due to the variability in duration of inhibition (280 sec. to 580 sec.) the percentage of total time is used.

a subminimal stimulus was observed to cause relaxation of the abdominal wall without detectable contraction of the diaphragm.

In a preliminary report (5), it was stated that cervical vagotomy on the right side abolished most of the response to stimulation. Subsequent observations have shown that the effects of unilateral vagotomy depend upon the position of the animal. If it is lying on the vagotomized side the period of respiratory arrest is greatly prolonged and the response to stimulation on the upper side is markedly reduced or abolished. Reversing the position of the animal in the middle of a period of respiratory arrest, so that the vagotomized side is up results in increased sensitivity to the reflex or in the immediate resumption of spontaneous breathing. Alternate periods of inhibition and spontaneous breathing can be produced by this means with great regularity. The periods of spontaneous breathing are frequently characterized by an early slowing of rate apparently by a process of adaptation. Figures 2 and 3 illustrate the type of record obtained with unilateral vagotomy and unilateral pneumonectomy respectively. Thus the threshold to stimulation and the duration of inhibition for a

given dose of pentothal in a unilateral vagotomized animal depend upon which side the animal is lying. Bilateral vagotomy abolished the reflex response and further prolonged the period of inhibition. The effects of unilateral vagotomy appear to persist indefinitely in the recovered unanesthetized animal; for example, the average

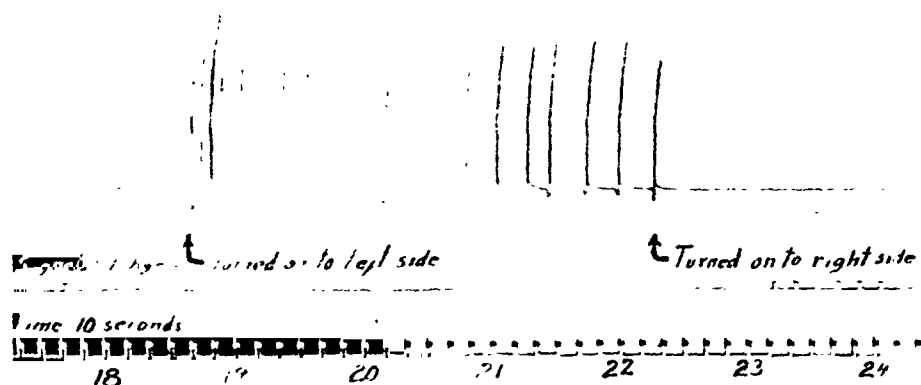


Fig. 2. RESPIRATION IN A DOG WITH A RIGHT UNILATERAL CERVICAL VAGOTOMY. The numbers under the time record are minutes since the last dose of nembutal (2.0 cc. of 2.5%). The state of anesthesia was such that the dog was not only in apnea but it was also refractory to 1 kg. stimulus when it was lying on its right side. On its left side, it breathed spontaneously.

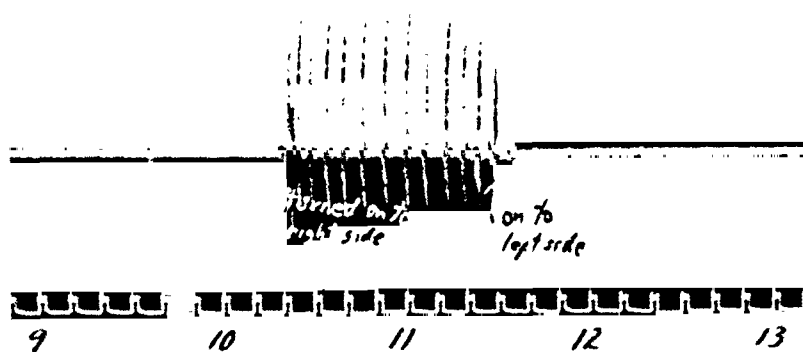


Fig. 3. RESPIRATION IN A DOG WITH LEFT-SIDED PNEUMONECTOMY. The numbers under the time record are minutes after the last dose of nembutal (1 cc. of 2.5%).

respiratory rate in a dog with left sided cervical vagotomy, about 4 months post operative, was 27 when lying on the right side, and 18 when lying on the left side.

The responses under deep pentothal anesthesia were studied in 6 dogs from two weeks to eight months following unilateral pneumonectomy. In each instance the pattern of response during pentothal inhibition followed that of unilateral cervical vagotomy, i.e., lying on the operated side depressed sensitivity to the reflex compared to the opposite position. The period of inhibition was also longer and spontaneous

breathing was slower. These animals also showed a marked difference in respiratory rate with change in position when unanesthetized. In a typical case, seven months postoperative, the rate was 21 when lying with the pneumonectomized side up compared to a rate of 15 when in the opposite position.

#### DISCUSSION

None of the above results confirms the suggestion of Draper and Whitehead (4) that the reflex is elicited by stretching the rectus abdominis and scalenus medius muscles by applying pressure at the point of their common insertion. Response to stimulation over a relatively large area of the thoracic wall and disappearance of response following bilateral vagotomy rather suggests that it is elicited by deformation of lung tissue exciting receptors of the Herring-Breuer reflex. Much of the rhythmicity of normal respiration is ascribed to this mechanism. The remarkable sensitivity of these receptors to deformation has been shown repeatedly. Adrian (7) found that impulses in pulmonary afferent fibers varied rhythmically with the heart beat when there was compression of adjacent lung tissue. Hammouda and Wilson (8) observed respiration in the anesthetized dog whose thorax had been enclosed in an air-tight box. Under these conditions an increase in pressure on the outside of the thorax of only a few mm. Hg increased the respiratory rate.

That such a reflex could operate under deep anesthesia is indicated by observations of Whitteridge and Bülbiring (9). Indeed these investigators think that many of the respiratory effects of various anesthetics are due to specific changes in excitability of the pulmonary stretch receptors (10). Further evidence for the ruggedness of this reflex is found in Adrian's report that discharge of pulmonary afferent fibers persisted as long as fifty minutes after cessation of circulation.

The responses to change in position described above may be explained tentatively in terms of lung deformation produced by the gravity shift of mediastinal structures such as the heart. In the intact animal lying on its side, the application of pressure to the thoracic wall causes deformation of the underlying lung tissue. The mediastinal structures, particularly the heart, tend to stretch the upper lung, but to compress the lung on the under side. Presumably the balance between excitatory and inhibitory influences is upset by the application of a mechanical stimulus to the chest wall which reduces the degree of stretch of the lung on the upper side, but exaggerates the retraction of the lower lung. Section of the vagus nerve on the upper side removes the inhibitory effects of the stretched lung allowing the excitatory effects of the lower lung to predominate. When the position of the animal is reversed by placing the vagotomized side down, the excitatory impulses from the lower lung are removed and inhibitory influences of the upper side predominate. A similar explanation may be applied to the results obtained with the unilateral pneumonectomized dog. The attempts of Anderson and Lindsley to demonstrate unilateral effects from lung receptors on the intercostal muscles were unsuccessful (11). Adrian however, reported changes in frequency of single afferent vagus fibers in response to change in position of the rabbit.

The results of the experiments reported here might indicate that, in the dog at least, a bilateral balance of opposing influences from the Herring-Breuer receptors



are normally acting upon the respiratory center. These effects are demonstrated by changing the position of the deeply anesthetized animal whose lung afferents have been sectioned on one side, or by observing the effect of position on the respiratory rate in the same animal when unanesthetized. The results also support the claim of Whitteridge and Bülbring that the Herring-Breuer reflex persists with deep anesthesia when other reflex mechanisms have been abolished.

Preliminary observations on man, following unilateral pneumonectomy, have shown little difference in respiratory rate with change in position during the post-operative convalescent period. Six out of nine patients examined had an average increase in rate of only 10 per cent when lying on the unoperated side. Two facts may account for this: the mediastinum is less flexible in man than in the dog and post-operative procedures to prevent mediastinal shift are commonly employed when pneumonectomy is performed in man. Change in position in one pneumonectomized patient under deep pentothal anesthesia produced slight changes in respiratory rate which were in the same direction as those observed in the experimental animal.

#### SUMMARY

The minimal pressure required for a reflex respiratory response during pentothal inhibition may be used to measure the rate of recovery of excitability. Reflex respiratory responses to light pressure on the chest wall in the deeply anesthetized dog are abolished by bilateral cervical vagotomy. Unilateral cervical vagotomy or unilateral pneumonectomy enhances the reflex response of the deeply anesthetized dog when lying with the operated side up; when the operated side is down the reflex response is depressed. The unilateral vagotomized or unilateral pneumonectomized dog has a faster respiratory rate when lying with the operated side up than in the opposite position. This difference in rate with change in position persists indefinitely in the unanesthetized animal.

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# PROPRIOCEPTIVELY INDUCED REFLEX PATTERNS<sup>1</sup>

G. N. LOOFBOURROW AND E. GELLHORN

*From the Department of Physiology, University of Minnesota Medical School*

MINNEAPOLIS, MINNESOTA

**L**ITTLE is known concerning the specific distribution of the peripheral effects of proprioceptive reflexes initiated by muscle stretch. A considerable portion of the available information is based on mechanical records of contraction. The present experiments were undertaken to determine by multiple electromyographic recording the response pattern in a selected group of arm muscles when one or several of them are stretched.

Lloyd (1) has conclusively demonstrated that the 2-neuron-arc myotatic reflex facilitates synergists of the muscle in which the afferent impulses arise and inhibits its antagonists. These effects are restricted to muscles acting at a single joint. Bineuronal reflex arcs, however, account for only a small fraction of the afferent fibers from skeletal muscle. Afferent impulses leading to multineuron reflex responses are almost certainly aroused by muscle stretch, for there is evidence of a spread of activity beyond the limits established by Lloyd for bineuronal reflexes. Denny-Brown (2) observed an excitation of the ankle extensor, soleus, in response to stretch of the knee extensor, quadriceps. Sherrington (3) demonstrated the response of certain contralateral limb muscles to stretch of the vastocruureus or the triceps surae.

Not only passive stretch, but also actively developed tension, results in proprioceptive reflex responses. These effects, too, extend beyond the limits of bineuronal representation established by Lloyd. For example, Cooper and Creed (4) showed that a proprioceptive reflex arising in the active tibialis anticus (upon stimulation of the appropriate ventral root) induced a contraction of the sartorius. Preventing the tibialis from shortening during its contraction, thus increasing its developed tension, resulted in a much stronger contraction of the sartorius. Gellhorn (5) has shown that proprioceptive reflexes arising in a muscle held under stretch during cortical stimulation augment the response (electromyograms of greater amplitude<sup>2</sup>) of its associated synergists at a neighboring joint, as well as its own.

Although Gay and Gellhorn have recently found that either passive stretch or actively developed tension of muscles (stimulation of a ventral root) excites the motor cortex (7), Gellhorn's observations show that the cortically induced response is altered only quantitatively and not qualitatively, by proprioceptive reflex facilitation. In other words, the *pattern* under the conditions of the experiments is determined by the site of cortical stimulation and modification of the response by proprioceptive reflexes is limited to alterations in the relative intensities of muscle response within the pattern. It may be assumed that if proprioceptive reflex activity is aroused in the absence of specific cortical stimulation the pattern of response will be a function of the spinal cord and not of the cortex. The following experiments show that proprioceptive impulses, set up by stretching a muscle, activate muscles in specific patterns similar to those found by Bosma and Gellhorn (8) to be elicited by cortical stimulation. These patterns include functionally associated muscles acting at neighboring joints.

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Received for publication July 15, 1948.

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

<sup>2</sup> It has been experimentally demonstrated that under cortical stimulation, E.M.G. amplitude is a reliable measure of the mechanical response (6).

## METHOD

Cats and monkeys were anesthetized with Dial-urethane (Ciba)<sup>3</sup>, 0.45 cc/kg., i.p. Potentials were led off by means of fine wires sewn into the muscles, amplified and recorded by an Offner crytograph. Drills were inserted in the humerus and ulna and fixation achieved by metal braces attached to the drills. Proprioceptive stimulation was accomplished either by passive movement of a joint or by a load applied to the tendon of a muscle. The biceps, triceps, flexor carpi (usually ulnaris) and extensor carpi (usually radialis) and the brachioradialis were routinely used.

## RESULTS

*Stretch of Elbow Extensors.* In a sensitive preparation, a slight flexion of the elbow (e.g., reducing the angle from 90° to 75°) leads to excitation of the triceps and flexor carpi. The flexor carpi responds even after it has been tenotomized and, therefore, is presumably not affected mechanically by the elbow movement. Moreover,

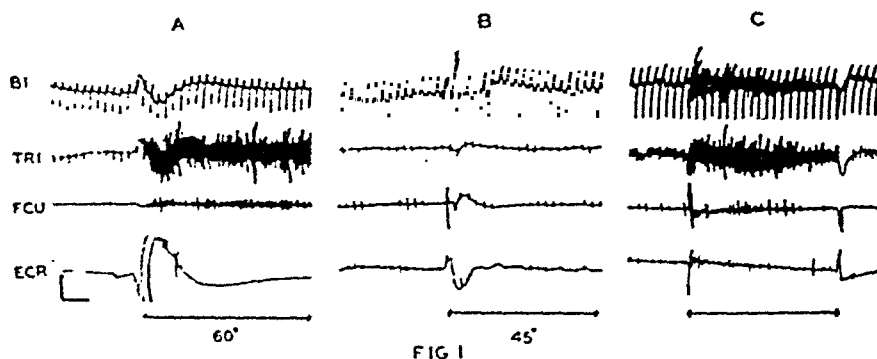


FIG. 1. RESPONSE TO ELBOW FLEXION before and after tenotomizing the triceps brachii (TRI) and the response to a pull on the triceps tendon. *Monkey.* A. The flexor carpi ulnaris (FCU) has been tenotomized. Elbow flexion excites the TRI-FCU complex. B. After triceps tenotomy, elbow flexion elicits no response. C. A pull on the triceps tendon excites the TRI-FCU complex. Co-contraction occurs in the biceps (BI), whose response is favored by an obtuse elbow angle (120°). Vertical axis = 60  $\mu$ V. In all figures (read from left to right) the horizontal axis equals 1 sec.

the response in the flexor carpi is as great as the maximal response obtainable by a direct pull on its cut tendon and this involves a pull of several hundred grams.

Figure 1 illustrates the effect of tenotomizing the triceps on the reflex response to elbow flexion. Before tenotomy of the triceps, flexing the elbow to 60° resulted in good responses in both the triceps and flexor carpi. This result persisted after tenotomy of the flexor carpi (fig. 1A). After tenotomizing the triceps also, even greater flexion elicited no response in either muscle (fig. 1B) although both still responded to a pull on the triceps tendon (fig. 1C). This shows conclusively that proprioceptive impulses arising in the triceps as a result of its passive stretch reflexly excite the flexor carpi.

Figure 1C shows that a pull on the tenotomized triceps induced a distinct E.M.G. in the biceps, although the reflex induced previously in the same preparation by flexion of the elbow failed to do so (fig. 1A). It is suggested that the different

<sup>3</sup> Kindly supplied by the Ciba Pharmaceutical Company.

behavior is due to the fact that the pull on the triceps tendon (fig. 1C) was exerted while the elbow was at an obtuse angle which is proprioceptively favorable to the biceps, whereas in figure 1A the triceps was proprioceptively excited through movement of the elbow to an acute angle which is unfavorable to the biceps. It was noted in an earlier study (5, 6) that proprioceptive reflexes in response to cortical stimulation occur preferentially in the stretched muscle. However, this induction of activity in the antagonist ('co-contraction') shows considerable individual differences which may in part be due to the degree of tonic activity (8). A rather strong co-contraction is illustrated in figure 2A. Flexing the elbow to  $45^\circ$  activates the triceps complex (TRI-FCU) although the flexor carpi is tenotomized and activates the biceps complex (BI-ECR-BR) to a lesser degree. That the activity of the latter is

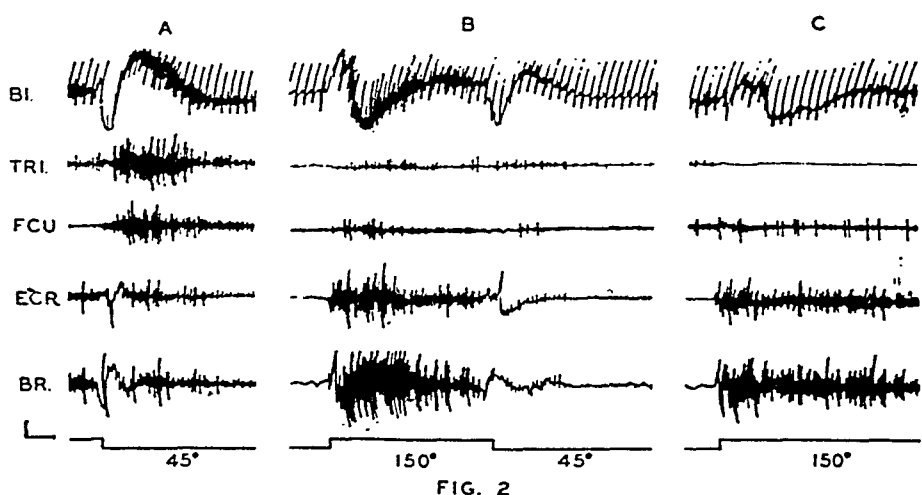


FIG. 2

Fig. 2. RESPONSE TO ELBOW FLEXION AND EXTENSION before and after tenotomizing the triceps and biceps, respectively. *Monkey*. The flexor and extensor carpi were previously tenotomized. A. Flexing the elbow to  $45^\circ$  activates the TRI and the tenotomized FCU. Co-contraction occurs in the biceps complex. B. After tenotomy of TRI in addition to the carpal muscles. Extension of the elbow elicits the same response as was observed prior to the tenotomy of TRI, i.e. activation of the biceps (BI), brachioradialis (BR) and the tenotomized extensor carpi radialis (ECR). Flexion now activates nothing, showing that the stretch reflex arising in TRI in A was responsible for the excitation of FCU and the co-contraction of BI. C. After tenotomy of BI also, elbow extension does not activate the BI, and the response of the associated ECR and BR is reduced. Vertical axis = 300 microvolts in channel 2, and 30 microvolts in all other channels.

a co-contraction dependent on the proprioceptive triceps reflex is shown by the fact that, after tenotomy of the triceps has abolished its own response to elbow flexion, the biceps complex is also silent (fig. 2B,  $45^\circ$ ). Summarizing our experience it may be said that the biceps and extensor carpi may be unaffected by elbow flexion, may exhibit a slight co-contraction, or may show an inhibition of tonic activity.

*Stretch of Elbow Flexors.* Extension of the elbow (fig. 2B) results in marked activity of the biceps, extensor carpi and brachioradialis. The response in extensor carpi radialis cannot be attributed to its being stretched by extension of the elbow, for its tendon had been cut. It therefore appears that its response is due to reflexes arising in other muscles which are stretched, such as the biceps and brachioradialis. The association of a response in the unstretched extensor carpi with one in the

stretched brachioradialis may be seen in figure 2C. The biceps had been tenotomized, therefore neither responded nor contributed to the response of the other muscles. The lesser response in the 'triceps complex' (triceps-flexor carpi) is rather insignificant (fig. 2B) and is best explained as a slight co-contraction (8, 5).

A load on the biceps tendon likewise elicits a strong biceps-extensor carpi response (fig. 3). In this case the angle of the elbow is fixed and there can be no suspicion of a direct mechanical effect on the extensor carpi.

*Stretch of Wrist Extensors.* In experiments with stimulation of the motor cortex, Gellhorn (5) found that the proprioceptive facilitation within a muscle complex may act in either direction, e.g., from biceps to extensor carpi, or the reverse. Apparently reflexes elicited by stretching a carpal muscle may induce similar reflex responses in specific upper arm muscles in the absence of cortical stimulation, since it was found

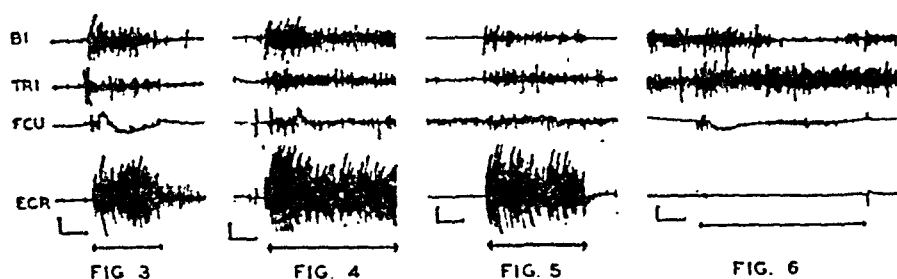


Fig. 3. RESPONSE TO A LOAD OF 500 G. ON THE BICEPS TENDON. *Cat*, all tendons intact. The response is primarily limited to the BI and its 'associated synergist' ECR. A lesser 'co-contraction' occurs in the antagonistic complex. Vertical axis = 100  $\mu$ v.

Fig. 4. RESPONSE TO VOLAR FLEXION of the cat's wrist to 80°. The principal response is in the ECR and its 'associated synergist' BI. The lesser response in the antagonistic complex is designated co-contraction. Vertical axis = 100  $\mu$ v.

Fig. 5. RESPONSE TO A 500 G. LOAD ON AN EXTENSOR CARPI in the cat. The chief response is in the stretched ECR. Note that the response of the BI is initially strong, in contrast to the gradual development of the 'co-contraction' in the TRI. Vertical axis = 100  $\mu$ v.

Fig. 6. RESPONSE TO A 500 G. LOAD ON THE TENDON of the flexor carpi in the cat. Note the excitation of the TRI and FCU. The tonic activity in the BI shows inhibition, during which the TRI-FCU response is augmented. Vertical axis = 30  $\mu$ v.

that volar flexion of the wrist excites the biceps-extensor carpi complex (fig. 4). The antagonistic complex may show co-contraction as in figure 4 or may be silent.

A load applied to an extensor carpi likewise excites the biceps. Figure 5, recorded in the same cat as figure 4, shows that even though the stretch is limited to only one extensor carpi, the biceps response is good. It will be noted that in this record the triceps and flexor carpi were tonically active before the load was applied to the extensor carpi. This tonic activity resulted from fixation of the wrist at 180° extension. Despite this fixation in a position favoring the triceps-flexor carpi complex, stretch of the extensor carpi (load) yielded primarily a biceps-extensor carpi response. Although the triceps response is fairly strong, it is generated slowly in contrast to an initially strong response in biceps. There can be no reasonable doubt that the primary response is that of the biceps-extensor carpi complex and that the activity in the triceps-flexor carpi complex is a co-contraction.

*Stretch of Wrist Flexors.* Stretching the flexors carpi by extending the wrist

may result in a triceps-flexor carpi response as described in the preceding paragraph (fig. 5, extreme left). However, this cannot be consistently demonstrated, presumably because this degree of stretch of the wrist flexors does not always generate sufficient impulses. It can be demonstrated, however, that even though wrist extension fails to elicit a response in triceps, the latter's response to stimulation of an appropriate afferent nerve is facilitated (unpublished observations). This is in agreement with Gellhorn's observation of proprioceptive facilitation under conditions of cortical stimulation. Moreover, direct application of a load to the tendon of a flexor carpi regularly results in a triceps response. In the experiment of figure 6 this reaction appears as an augmentation of tonic activity in the triceps. The antagonistic complex may show nothing, a co-contraction, or an inhibition. In figure 6 the biceps shows initially a slight co-contraction, followed by inhibition during which the activity of both triceps and flexor carpi is slightly augmented.

#### DISCUSSION

This study has shown that reflexes of proprioceptive origin result in patterns of muscular coordination identical with those resulting from cortical stimulation. The myotatic reflex arising in a single muscle has been shown to excite not only this muscle and its synergists at the same joint, but 'associated synergists' acting at a neighboring joint as well. It was frequently noted that even greater activity could be evoked in a muscle by impulses arising proprioceptively in other muscles than by autogenous proprioceptive stimulation. Antagonists frequently show the inhibition expected from reciprocal innervation, but also frequently show co-contraction. This is not surprising, since fixation of a limb, when it involves the stretching of antigravity muscles as in the 'supporting reaction', leads to simultaneous contraction of flexors and extensors.

The complete agreement between the proprioceptive reflex patterns described in this paper and the modification of motor response through fixation in cortically induced movements suggests that the same mechanism is involved in both sets of experiments. Apparently postural changes (fixation of a joint, etc.) modify the effects of stimulation of the motor cortex through proprioceptive reflexes, but whether this modification is exclusively spinal or involves supraspinal mechanisms has not been investigated. Further experiments on the persistence of these specific reflex patterns of the stretch reflex after spinal transection, as well as the influence of deafferentation of a limb on the pattern of movement elicited by cortical stimulation, may clarify these questions.

Although these experiments have been restricted to a few muscles, the results suggest that any muscle of a limb is potentially subject to some regulation by proprioceptive impulses arising in all other muscles of that limb and that this mechanism is of great importance in muscle coordination no matter whether movements are elicited by reflex or by cortical mechanisms.

In view of these results, the observation that in certain cases of poliomyelitis a muscle which cannot be made to contract by voluntary effort may be excited by passively stretching another muscle, even an antagonist (9), or by a voluntary effort to contract other muscles (unpublished observations), appears less paradoxical. These

and related data (10) should be taken into consideration in a program of muscle reeducation.

#### SUMMARY

A study of proprioceptive reflexes is reported in which the effect of passive movements of the elbow and wrist joint or the action of pulling the tendon of an individual muscle is investigated by means of electromyograms. The experiments were performed on anesthetized cats and monkeys with the following results:

1. Passive movements of the elbow lead mainly to activity in the triceps and flexor carpi on flexion and to activity in the biceps and extensor carpi on extension. These effects persist even after tenotomy of the carpal muscles, but are abolished by tenotomy of the biceps and triceps. In the latter case the application of a load to the triceps again induces activity in the triceps complex (triceps-flexor carpi).

2. Stretching of wrist muscles by passive movements or by loading of an individual muscle induces activity in the upper arm muscles. Here again the effect is specific inasmuch as stretching of the flexor carpi induces activity in the triceps while loading the extensor carpi induces activity in the biceps.

3. If through passive movements or muscle stretch a considerable activity appears in the stretched muscle and in a specifically associated muscle (e.g., stretch of biceps causing activity in biceps-extensor carpi and stretch of triceps causing activity in the triceps-flexor carpi) the effect may also appear, but in lesser degree, in the antagonists of these muscles. This phenomenon is designated co-contraction.

4. It is emphasized that the same specific muscle patterns established earlier by Bosma and Gellhorn in experiments on stimulation of the motor cortex are produced by the myotatic reflex. The latter has a specific but less restricted effect than has been assumed heretofore.

5. Co-contraction as the result of the myotatic reflex shows the same features as seen in experiments with stimulation of the motor cortex.

6. The importance of this newer knowledge of the myotatic reflex for the problem of muscle reeducation is emphasized.

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# RÔLE OF CARBON DIOXIDE AND OF THE HINDBRAIN IN AGENE-INDUCED CANINE EPILEPSY<sup>1</sup>

MAURICE L. SILVER<sup>2</sup> AND GEORGE H. POLLOCK<sup>3</sup>

*From the University of Illinois, College of Medicine*

CHICAGO, ILLINOIS

NEWELL, Erickson, Gilson and Elvehjem (1) showed that dogs on a wheat gluten diet developed electroencephalographic patterns similar to those seen in human epilepsy. These changes could be detected as early as three days after the diet started and prior to the onset of clinical signs. In a previous paper (2), this group described the electroencephalographic changes as high voltage slow waves with spiking. When they were fortunate enough to get an animal to have a seizure during a recording of the EEG, the typical picture appeared. Mellanby (3) first noted that bread made from flour treated with nitrogen trichloride caused convulsions in dogs. Silver, Monahan, Klein and Pollock (4) demonstrated that wheat protein treated with varying amounts of nitrogen trichloride was the responsible agent for the abnormalities in the EEG. The first changes seen were an increase in the amplitude of the cerebral potential which fused with an increased frequency. A day later high voltage-low frequency waves appeared. This picture was well established by the end of three days of diet and continued throughout the course of the intoxication. There were sometimes spikes or spike and dome formations. Spontaneous seizures usually appeared after five days of diet, but if the amount of nitrogen trichloride added was increased, these seizures occurred earlier. The seizures were characterized by a 'slow build up' to a full discharge of the cerebrum. Then the characteristic iso-electric post-ictal period followed. Similar changes in the EEG have been observed in dogs fed gliadin, glutenin, lactalbumin, casein or amino acid mixtures when these had been treated with nitrogen trichloride (5).

In animals that have typical preconvulsive EEG's, convulsions can be induced by the inhalation of 20 per cent carbon dioxide and 80 per cent oxygen. The abnormalities so produced can first be detected from pick-up leads over the cerebellum.

## EXPERIMENTAL METHODS

These experiments were conducted entirely on dogs that had been fed an adequate diet (6) containing flour treated with nitrogen trichloride (30-100 gm/100 lb.

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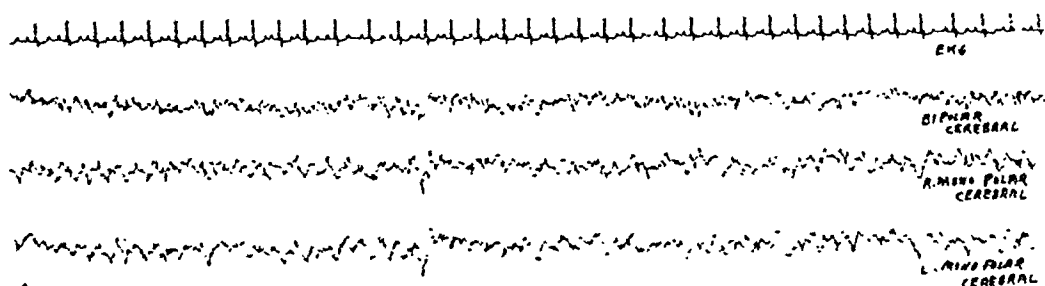
Received for publication June 16, 1948.

<sup>1</sup> Paper presented before American Branch International League Against Epilepsy, June 13, 1948, Atlantic City, N. J.

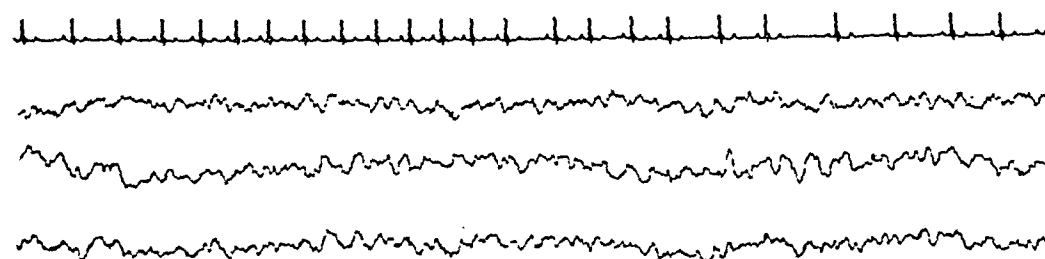
<sup>2</sup> Present address: The Johns Hopkins Hospital, Baltimore, Maryland.

<sup>3</sup> Present address: Illinois Neuropsychiatric Institute, Chicago, Illinois.

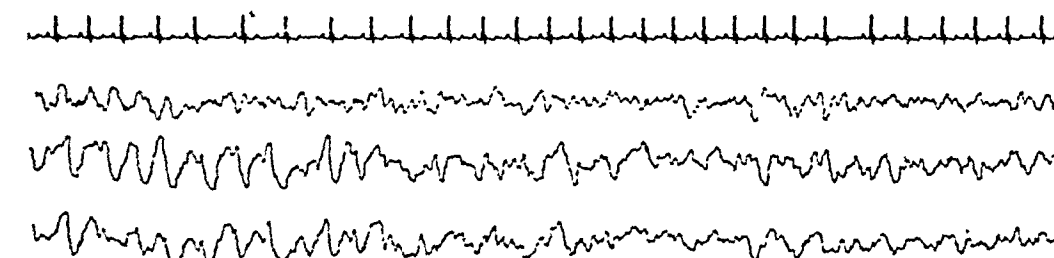




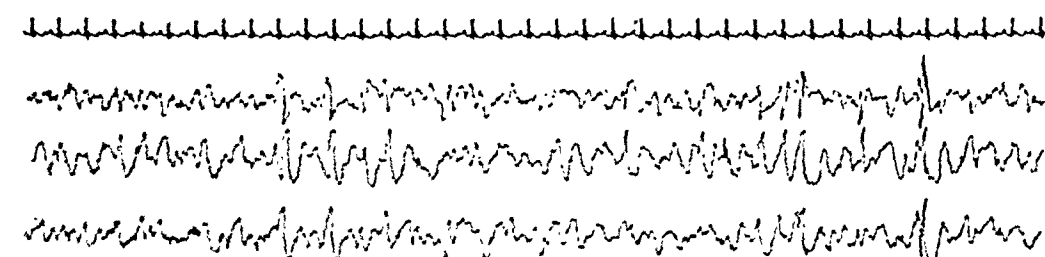
**A** TYPICAL PRE- $\text{CO}_2$  RECORD



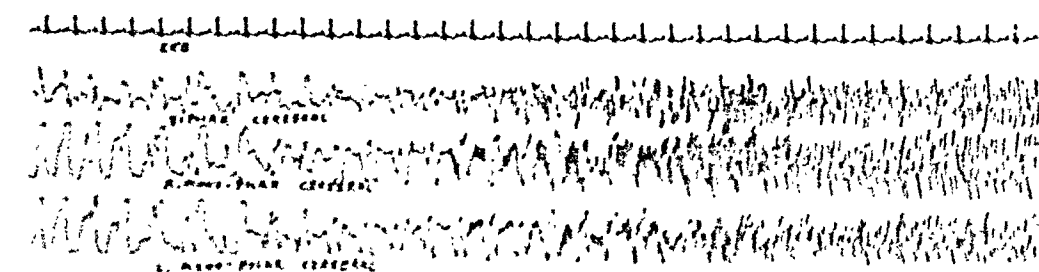
**B** 10%  $\text{CO}_2$  + 90%  $\text{O}_2$  in 10 sec.



**C** 10%  $\text{CO}_2$  + 90%  $\text{O}_2$  in 1 min.



**D** 10%  $\text{CO}_2$  + 90%  $\text{O}_2$  in 2 min.



EMG 10%  $\text{CO}_2$  + 90%  $\text{O}_2$  off  
(Time approximately 3 min.)

**E**

1—  
100

Fig. 1. A. Typical pre- $\text{CO}_2$  record. High voltage, slow waves with an occasional spike. B. Inhalation of 10%  $\text{CO}_2$  plus 90%  $\text{O}_2$  for 10 seconds. High voltage, slow frequency, begins to change state for position. EEG irregular and slower. C. Inhalation of gas mixture for one minute. Maximal high voltage, slow frequency seen. D. Inhalation of gas mixture for 2 minutes. Spikes now seen more frequently interspersed between high slow waves. E. Renewal of  $\text{CO}_2$  mixture after 3 minutes. A return to low voltage seen thereafter.

of flour). After several days' observation the animals were given the experimental diet. They were observed daily and, after various times during which known quantities of diet had been ingested, were used for acute terminal experiments.

A tracheal cannula was inserted and one femoral vein was exposed under ethyl ether anesthesia. Paralysis was induced with 25 mg. of dihydro-B-erythroidine hydrobromide<sup>4</sup> intravenously and artificial respiration started (50-75 cc/stroke, 10-15 strokes/min.). A continuous intravenous drip of a solution containing 1 mg/ml. of dihydro-B-erythroidine hydrobromide in 0.156 M sodium chloride was introduced into the exposed femoral vein. Electrodes were screwed into the skull overlying the right and left parietal cerebrum and over the cerebellar hemispheres. EKG was recorded from leads in the right fore and left hind limbs. The Grass six-channel electroencephalograph was used throughout. The mixture of carbon dioxide and oxygen used was commercially prepared. The respirator is so designed that a gas can be rapidly substituted for room air when desired.

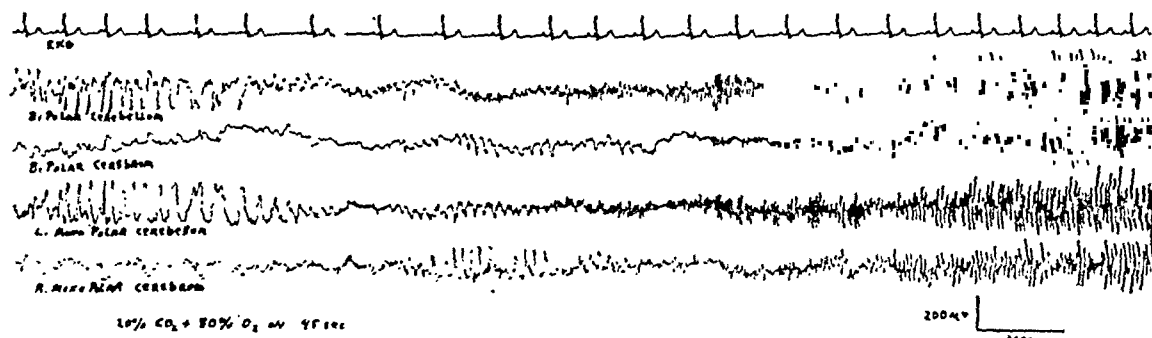


Fig. 2. TWENTY PER CENT  $\text{CO}_2$  plus 80%  $\text{O}_2$  inhaled for 45 seconds. Spikes and high slow waves first appear in cerebellar leads. Activity spread to cerebral leads. Seizure starts in cerebellar leads and after a short delay spreads to cerebrum. EKG is irregular and slow.

An initial period of about 30 minutes was allowed for stabilization of the electroencephalographic record. Thereafter a mixture of 20 per cent carbon dioxide with 80 per cent oxygen was introduced into the respirator and the animals ventilated for 3 minutes. A 30-minute interval elapsed before the gas was again employed.

Control experiments were conducted on dogs fed an identical diet containing unagenized flour.

## RESULTS

The typical abnormalities induced by ingestion of agenized (nitrogen trichloride treated) flour appeared first in the cerebellar leads and later in the cortical leads. They could be intensified or a seizure induced by the inhalation of the mixture (fig. 2). The fit usually appeared during the beginning or shortly after the end of the inhalation (fig. 1). Normal animals showed no seizures with inhalation of the mixture. However, they did show reversible changes; an increase in frequency and decrease of amplitude of the EEG and some slowing and irregularity of the EKG.

<sup>4</sup> The authors wish to thank Merck and Company who kindly supplied this drug.

## DISCUSSION

Silver (7) has reported definite pathology in the Purkinje layer of the cerebellum and the dentate nucleus of dogs fed flour treated with nitrogen trichloride. It is very possible that the activity of the hindbrain is a reflection of this pathology.

The synergistic action of carbon dioxide is as yet unexplained. Roseman, Goodwin and McCulloch (8) have shown that inhalation of 20 to 25 per cent carbon dioxide in oxygen results in a slowing of the heart rate and an increased frequency with decreased amplitude of the EEG in cats. They note that the oxygen tension of the cortex is also elevated. Dusser de Barenne, McCulloch and Nims (9) found that hypoventilation (increased carbon dioxide tension) shifts the  $pH$  of the cerebral cortex to the acid side. This low  $pH$  is associated with low electrical activity. Gibbs, Gibbs and Lennox (10) have shown that increasing the carbon dioxide tension of arterial blood causes a specific dilatation of the cerebral vascular bed with a resultant increase in the cerebral blood flow. It may well be that the increased blood flow through the brain results in a greater concentration of the toxic agent and this may be sufficient to intensify existing abnormality or precipitate actual seizure activity. Why this should occur when the gas mixture is initially administered or immediately after its removal is still unanswered. It may be due to rapid shifts in the brain  $pH$  or oxygen tension. The specific inhibitory effect of carbon dioxide on the cerebral cortex may allow subcortical structures activated by the toxic agent to manifest themselves. No definite interpretation is offered at present.

## SUMMARY

Inhalation of 20 per cent carbon dioxide and 80 per cent oxygen is synergistic with the toxicity induced by the ingestion of flour treated with nitrogen trichloride. It accentuates existing electroencephalographic abnormalities and can precipitate a seizure in a susceptible animal. These are seen first in leads over the hindbrain and characteristically when the inhalation starts or shortly after it is terminated.

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# ADAPTATION TO EXPERIMENTAL MOTION SICKNESS IN DOGS<sup>1</sup>

R. L. NOBLE

*From the Department of Medical Research, University of Western Ontario*

LONDON, ONTARIO

SINCE 1942 the subject of experimental motion sickness has been studied extensively in this department. As only a few papers had been published on motion sickness in animals prior to this time (1, 2), it was essential to study the factors which influenced the susceptibility of the animal. It was found (3) that dogs were a more suitable species than cats since some 80 per cent were found to vomit after motion on a simple swing. By altering the extent of swinging it was possible to divide susceptible animals into three groups according to the ease with which they could be made to vomit. The most highly susceptible dogs were found to show an extremely consistent response to swinging even though they were used over a three- to four-year period. Of the different component motions of a swing none was as effective individually as the composite action. Changes in horizontal acceleration were the most effective stimuli, vertical motion being relatively ineffective. Animals which were susceptible to swinging were also found to become ill after driving in a motor truck and after exposure to motion in a small boat in rough water. Since the initial project was to find drugs which might serve to alleviate the symptoms of motion sickness, methods for assaying possible agents were worked out on dogs (4).

A large series of compounds, chiefly barbituric acid derivatives, were subsequently tested for their ability to prevent motion sickness in dogs (5). Many of these substances were found to possess this property which appeared to act specifically against motion sickness since this effect was not related to the hypnotic or anesthetic action of the compound. The response of human beings to treatment with a number of barbiturates was also tested against motion sickness produced by swinging (6). One compound V-12, ethyl  $\beta$ -methyl allyl thiobarbituric acid<sup>2</sup> was tested by various workers on sea trials and was found to be an effective therapeutic agent. The results of the Canadian trials with various drugs have been reviewed by Noble, Sellers and Best (7).

During the five years in which a colony of some 25 susceptible dogs was maintained for these various experiments, changes in susceptibility to swinging due to adaptation were noted. The results described in this paper are a summary of these

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Received for publication July 2, 1948.

<sup>1</sup> Most of the experiments recorded were carried out at the Research Institute of Endocrinology, McGill University, Montreal, P.Q.

<sup>2</sup> Supplied by Abbott Laboratories, North Chicago, Ill. and now prepared under the name of 'Mosidal'.

observations and some additional experiments designed to obtain more information on the subject of adaptation to motion.

#### METHODS

Dogs of various types, ages and sex susceptible to motion were selected at random for these experiments. They were usually of from 5 to 10 kg. in weight. Except during the experiment they were housed in a separate building and had no contact with the swings. Each animal was fasted for 19 hours but immediately before swinging was fed a small meal of minced meat. Dogs which did not vomit following 45 minutes in the swing were considered to be immune or protected in therapeutic experiments. The swing used was electrically driven with a radius of  $14\frac{1}{2}$  feet. It passed through an angle of  $90^\circ$  and had a frequency of 15 complete swings per minute. To determine different degrees of susceptibility the angle of swinging could be reduced. Dogs which vomited when the angle was reduced to  $22\frac{1}{2}^\circ$  were considered highly susceptible while those which vomited when swung through an angle of  $45^\circ$  but not  $22\frac{1}{2}^\circ$  were classed as moderately susceptible. In order to minimize adaptation, which was expected to occur, in early experiments animals were not used more frequently than once a week. For the last three years of the study, however, the dogs of the moderately and highly susceptible groups were used at intervals of every five days except during the usual holiday periods. In one experiment a dog was exposed to vertical motion, by a system of pulleys and ropes; the swing was used to drive the apparatus as previously described (3).

#### RESULTS

If one considers the effect of repeated exposures to motion on all of the dogs which have been observed over periods of years, there is usually a definite decrease in susceptibility of the animal. This adaptation effect probably occurs in every animal although the degree varies from an animal becoming totally immune to one which requires a slightly increased time of swinging before vomiting. Even when the dogs were used only at weekly intervals, some 30 per cent of the animals of the least susceptible group (i.e. those vomiting only when swung through an angle of  $90^\circ$ ) over a two-year period become refractory to swinging. Others required longer periods of swinging before becoming ill. Such an effect rendered low susceptible animals of this type unsatisfactory for therapeutic experiments where a consistent control response was essential. Moderately or markedly susceptible dogs, even though used at intervals of only five days, seldom became immune to motion although some of them showed a decrease in susceptibility. The response of the majority of the susceptible animals remained practically unchanged over a number of years. One dog of this type was obtained in June 1943. The first 10 times the animal became ill it averaged 6 minutes on the swing. Three years later on 10 consecutive swingings at 5-day intervals the same dog averaged 10.5 minutes for its time of vomiting. Over this total period the animal vomited on 100 occasions and even though used for protective therapeutic experiments the average time of vomiting was 11.6 minutes.

*Influence of the Interval between Swinging on Adaptation.* A number of dogs

were swung at different intervals of time to test their susceptibility. These animals had been susceptible and showed a positive response to swinging for two to three years before these experiments were started. A brief protocol of the history of each animal is added to the tables. The effect of swinging at weekly intervals is shown in *dog 66* which had developed increasing adaptation to motion. Five other dogs required swinging at more frequent intervals to show this phenomenon. In these experiments the swing was stopped as soon as the dog vomited (table 1).

It is seen that the 5 dogs listed all showed adaptation to motion since the time taken to vomit and the incidence of vomiting tended to become gradually reduced. In the case of *dog 66*, swinging at weekly intervals was effective whereas *dogs 47* and *63* required repeated swinging twice weekly for a considerable period to develop resistance. The animals used daily or twice daily become immune rapidly. Such treatment apparently was cumulative and had a long lasting effect since the adaptation occurred gradually and was still present after rest intervals of 6 to 10 weeks. *Dog 27* became totally resistant and *dog 63* nearly so after the repeated exposures to motion. In three cases animals were compared at different occasions using different intervals between swinging. It can be noted that *dogs 25* and *63* became adapted much more rapidly when swung daily than when swung at 2- or  $3\frac{1}{2}$ -day intervals. Similarly *dog 67* showed a more rapid response when swung twice daily.

*Influence of the Duration of Swinging on Adaptation.* Three dogs were exposed to motion for a definite period of time, irrespective of whether or not they vomited, until they became adapted to motion. The results were then compared with those obtained after a suitable rest period by allowing the dogs to adapt as described in the preceding section. The time interval between swing tests in this case was kept constant. In many cases the animals became ill repeatedly (table 2). It would appear from those results that, in the case of *dog 60*, adaptation was accomplished more rapidly in the experiments where swinging was continued for 45 and 60 minutes, although in the other animals there was little difference. Unfortunately, the general tendency for immunity to develop, as noted previously, makes it difficult to assess changes in the same animal and, in many cases where swinging was stopped after vomiting, the times actually approximated 45 minutes. *Dog 42*, which was swung twice daily, showed a gradual increasing immunity so that at first vomiting occurred twice on the morning swing and once on the evening swing. As adaptation increased, vomiting took place only once on each time and then only in the morning until finally immunity was present.

*Adaptation to a Different Type of Motion.* One dog only was found to vomit consistently when exposed to vertical motion of a distance of eight feet four inches (double that occurring during ordinary swinging). It seemed of interest to test whether or not the adaptation to vertical motion would afford protection on the swing and vice versa. Experiments on this animal are listed in table 3. In two tests the animal was swung 45 minutes irrespective of whether vomiting occurred.

In the first test it may be seen that *dog 68* became adapted to the swing and remained so. However, on the sixth day it vomited in 35 minutes when exposed to vertical motion. In the third test the animal became immune to vertical motion after five daily runs and on the seventh day did not vomit when tested on the swing.

It seems therefore that adaptation to both types of motion was accomplished on one occasion but not on the other. The other tests on this animal shown in the table are typical of the increasing adaptation as previously described.

*Adaptation under Drug Therapy.* Some experiments were designed to see whether certain substances would inhibit or enhance the development of adaptation

TABLE 1. ADAPTATION TO MOTION AFTER SWINGING AT DIFFERENT INTERVALS OF TIME

Dog 66				
DAYS .....	1	7	14	21
	Time before vomiting, min.			
Initial test.....	25	45	N	N
After 4-wk. rest.....	25	40	N	N
" 2 " ".....	14	N		
" 2 " ".....	19	N		

Dog 25									
DAYS .....	1	2	3	4	5	6	7	8	9
	Time before vomiting, min.								
Initial test.....	40	40	N	N					
After 4-wk. rest.....	40		35		40		N		N

Dog 27									
Initial Test .....	40	N	30	N	35	N	N	N	
After 3-wk. rest .....	25	N	N						
" 6 " ".....	N	N							
" 10 " ".....	N	N							

Dog 67									
DAYS .....	1	1	2	2	3	4	5	6	
	Time before vomiting, min.								
Initial test .....	40	40	N	N	N				
After 10-wk. rest .....	20	—	25	—	40	40	N	N	

Dog 47										
DAYS .....	1	3	7	10	14	17	21	24	28	31
	Time before vomiting, min.									
Initial test .....	22	22	33	15	33	N	43	N	35	N
" ".....	18	19	10	25	19	20	22	24	25	25

Dog 63										
DAYS .....	15	18	42	45	49	52	56	59	61	66
	Time before vomiting, min.									
Initial test .....	N	N	35	N	37	N	N	N	N	N
" ".....	22	32	N	N	36	30	30	42	N	N

Dog 63 (cont'd)

DAYS .....	1	2	3	4	5	6
After 2 wk. rest.....	25	30	30	40	N	N
" 3 " " .....	35	N	N			
" 8 " " .....	N	N				
" 10 " " .....	40	N	N			

N = not ill in 45 min.

Dog 66 Moderately susceptible for previous 2 yrs., changing to low susceptibility before start of above experiment.

Dog 25 After 2 yrs. of testing had changed from moderate to low susceptibility; above experiment 1 yr. later.

Dog 27 Moderately susceptible throughout, used for 3 yrs. previous.

Dog 67 After 1 yr. of testing had changed from moderate to low susceptibility; above experiment 7 mo. later.

Dog 47 After 9 mo. of testing had changed from moderate to low susceptibility; experiment 11 mo. later. Dog 63 Moderately susceptible throughout; used for 2 yrs. previous.

to motion and in one case an animal was treated with V-12 to see whether or not immunity would develop even though the symptoms of motion sickness were prevented. Dog 47, swung daily, also received .5 cc. of prostigmine 1 in 4000 subcutaneously. Adaptation occurred in the same time as in a previous test where no therapy was given. Dog 44 received daily 20 mg/kg. orally of allyl (1-methyl butyl) imino thiobarbituric acid, a substance which appeared to increase the susceptibility of dogs to motion sickness. Adaptation under such treatment took place in the expected normal fashion. Dog 63 received daily injections of 0.4 mg. hyoscine hydrobromide and was swung daily. Immunity developed as in control tests, such treatment apparently neither increasing nor preventing adaptation.

Dog 47 was treated daily for 11 days with 10 mg/kg. orally of V-12; during this period the animal was swung twice weekly. Such treatment prevented vomiting and all symptoms of sickness on each occasion and immunity had developed when therapy was stopped. The same animal was similarly treated for 11 days with V-12 after it was adapted and swinging continued. After cessation of treatment the animal was still immune even though swinging was continued for 65 minutes. Apparently effective therapy with V-12 did not prevent adaptation from taking place nor did it interfere with its continuation after it was once established.

*Conditioned Reflex to Motion.* In initial experiments it was believed that dogs susceptible to motion might readily become conditioned so that they would vomit at the sight of the swing or the container in which they were secured to it. When animals were swung every seven or five days even though this was continued over a period of years, no case of conditioning was ever encountered. In the 10 animals which were used to demonstrate adaptation, there was also no sign of conditioning even though some dogs were swung twice daily. In all cases the trend was for animals to become refractory to swinging rather than the reverse. Recently Dr. W. H. Johnston of the Zoology Department of this University made observations on a dog susceptible to motion which had become conditioned to the swing so that at the sight of it salivation and vomiting would occur. This animal was treated with V-12 and



TABLE 2. ADAPTATION TO MOTION AFTER SWINGING FOR DIFFERENT LENGTHS OF TIME

<i>Dog 60</i>									
DURATION OF SWINGING, MIN.	1 DAY	3 DAYS	7 DAYS	10 DAYS	14 DAYS	17 DAYS	21 DAYS	24 DAYS	28 DAYS
	Time before vomiting, min.								
Until vomiting.....	20	15	15	15	13 10	35	N	N	
45.....	30	N	30	N	25 32	N	20	N	N
60.....	32 35 60	N	58	N	N				
Until vomiting—after 8-wk. rest.....	35	35	N	N					
Until vomiting—after 10-wk. rest.....	N	N							

<i>Dog 44</i>										
	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	8 DAYS	9 DAYS	10 DAYS
	Time before vomiting, min.									
45.....	25	N	45	38	18	35	25 42	25	N	N
45—after 3-wk. rest.....	40	30	15	25	N	N				
Until vomiting—after 6-wk. rest.....	40	45	35	N	N					
Until vomiting—after 10-wk. rest.....	26	25	25	23	23	25	30	N	N	

<i>Dog 42</i>												
	1 DAY	1 DAY	2 DAYS	2 DAYS	3 DAYS	3 DAYS	4 DAYS	4 DAYS	5 DAYS	5 DAYS	6 DAYS	7 DAYS
	Time before vomiting, min.											
45.....	20 35	40	30 30 40	N	20	N	20	45	40	N	N	
45—after 3-wk. rest.....	30		35		40		25		N		N	
Until vomiting—after 6-wk. rest.....	40		45		35		N		45		N	N
Until vomiting—after 10-wk. rest.....	35		N		N							

N = not ill in 45 min. or time indicated.

*Dog 60.* After 6 mo. of testing had changed from moderate to low susceptibility; above experiment 18 mo. later. *Dog 44.* Moderately susceptible throughout; used for 22 mo. previous.

*Dog 42.* After 1 yr. of testing had changed from moderate to low susceptibility; above experiment 10 mo. later.

curiously enough its symptoms due to conditioning were prevented as was vomiting after swinging. Whether this animal could be made to become adapted to motion

while being swung under V-12 therapy would be of interest since one might expect unconditioning to occur under these circumstances.

TABLE 3. ADAPTATION TO SWINGING AND VERTICAL MOTION

Dog 68								
DURATION OF SWINGING, MIN.	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS	7 DAYS	8 DAYS
	Time before vomiting, min.							
45.....	(20)	40	{ 18 30}	N	N	(35)	N	N
45—after 3-wk. rest.....	40	35	40	N	40	N	N	
45— “ 6 “ “ .....	17	(10)	(N)	(35)	(N)	(N)	N	
Until vomiting—after 10-wk. rest.....	21	25	30	30	N	N		

N = not ill in 45 min. Brackets indicate that vertical motion was used.

Dog 68 Highly susceptible at start for 5 mo., moderately susceptible for 9 mo., low susceptibility for 6 mo. before experiment.

#### DISCUSSION

The results reported in this paper show that the dog may readily become adapted to motion sickness. Over a period of years during which a colony of susceptible animals was used for repeated tests there was a gradual change so that the susceptibility diminished. This was shown in many cases by an increased degree of swinging necessary to make the dog vomit. Animals which showed the lowest susceptibility at the start become immune to motion much more rapidly than those of highest susceptibility. Of this latter group the response of some animals remained practically unchanged when swung every five days for as long as three years. The gradual alteration in susceptibility which takes place in dogs is of particular importance when one is conducting therapeutic experiments. Besides an alteration in the control test, animals as they become less susceptible to motion are effectively treated by progressively smaller doses of drugs (4). The details of experiments to show adaptation have been given in the accompanying tables. The 10 dogs shown all became relatively resistant to swinging after repeated exposures. In one case with intervals of one week between tests the animal became refractory. In general the shorter the interval between tests the more rapidly did adaptation take place. Even with rest intervals up to 10 weeks between tests, however, the animals all showed a progressive loss of sensitivity. Some tests were designed to see if prolonged swinging on each occasion would enhance the onset of refractiveness. In one case this seemed to be so but the changing susceptibility of the dogs to repeated tests made the observations unreliable.

In the human being adaptation to any one form of motion appears to be highly specific in that individuals became ill when exposed to other forms of motion (8). One dog which was normally susceptible to vertical motion remained so although it was made refractory to the ordinary swing. On the other hand when it was made immune to vertical motion it was found to be also immune to swinging. Apparently

these two types of motion were sufficiently similar so that adaptation was effective to some extent in both. Various drugs were administered to see if they would affect the development of resistance in any way. Two substances, prostigimine and a thiobarbiturate, which might be expected to render the dogs more susceptible to motion sickness had no effect on adaptation to motion. Hyoscine, which is effective in treating motion sickness in human beings but not in dogs, did not enhance or prevent the onset of adaptation. The barbiturate V-12 was used to prevent motion sickness in a susceptible dog and this animal was able to become adapted to repeated swings even though it was not ill. Also the refractory state was maintained by swinging when the animal was still protected by treatment. This experiment would suggest that effective therapy of motion sickness is compatible with the development of adaptation to motion. Throughout the experiments on repeated exposure to motion no animal showed any evidence of becoming conditioned to vomiting. One such animal is reported from a different laboratory. The salivation and vomiting which normally took place when this dog was simply placed on the swing was prevented with V-12 as was any effect of actual swinging.

#### SUMMARY

Dogs which have been used for repeated tests on motion sickness show a gradual adaptation to motion on a swing. This refractoriness varies in extent, but is most readily produced in dogs having low initial susceptibility to motion. On the other hand, highly susceptible animals may show only little change to swinging every five days over a three-year period.

Although swinging every seven days may cause adaptation, the state rapidly follows daily or twice daily swingings. The speed of development is apparently proportional to the number of exposures. Some evidence is presented that adaptation occurs more rapidly when the duration of swinging is increased. Treatment with prostigimine or hyoscine did not inhibit or enhance adaptation to motion. Adaptation to motion took place and was maintained even though a dog was effectively treated by the barbiturate V-12. Conditioned vomiting to the swing was not observed. One animal of another worker showed this reaction which was prevented by treatment with V-12.

This research was supported by grants from the National Research Council, Ottawa. Mr. Pellesen gave valuable technical assistance in the experiments. The author wishes to thank Dr. J. B. Collip for his continued interest in this problem.

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# EFFECT OF ELECTRICAL STIMULATION UPON ATROPHY OF PARTIALLY DENERVATED SKELETAL MUSCLE OF THE RAT<sup>1</sup>

A. J. KOSMAN, E. C. WOOD AND S. L. OSBORNE

*From the Department of Physiology and Physical Medicine, Northwestern University Medical School*

CHICAGO, ILLINOIS

ALTHOUGH considerable experimental data have been accumulated concerning the effects of electrical stimulation upon the atrophy of totally denervated skeletal muscle (1-8), there is no direct information of the behavior of partially denervated muscles toward such treatment. And yet, in its clinical applications, electrotherapy of paralyzed muscles involves muscles whose denervation is often incomplete. The present studies were undertaken to obtain such experimental evidence.

## METHODS AND MATERIALS

The animals used throughout these experiments were adult male albino rats weighing from 275 to 350 gm. The muscle investigated in all cases was the gastrocnemius. In the rat, this muscle receives its major motor innervation from the fourth and fifth lumbar nerves with a minor contribution from the sixth lumbar nerve (9, 10).

By selective bilateral lumbar nerve section three degrees of denervation of the gastrocnemius were produced: moderate, severe and complete. At the end of the experimental period supra-maximal electrical stimuli were applied to the sciatic nerve (indirect stimulation) and to the gastrocnemius directly (direct stimulation) and the tension developed by the left and right muscles upon such direct and indirect stimulation was measured. The animals were then killed and wet weights of the muscle obtained. Tension measurements were made by a torsion myograph of the Blix type. Among those rats selected for treatment, the gastrocnemius on one side was stimulated once daily for the entire period of denervation with a modulated sinusoidal current having a carrier frequency of 25 cycles per second. The carrier frequency was sinusoidally modulated at a rate of 25 per minute. The stimulating electrodes were applied directly to the skin overlying the gastrocnemius.

The procedure for the daily stimulation was as follows: A 30-second period of stimulation followed by a 10-minute rest period and an additional 30-second period of electrical exercise. The animals were divided into the following experimental groups:

- Group 1.* Moderate partial denervation. Bilateral section of the fifth lumbar nerve.  
a) 21 rats. No treatment. Sacrificed 14 days following nerve section.

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Received for publication August 4, 1948.

<sup>1</sup> This work was aided by a grant from The National Foundation for Infantile Paralysis, Inc.

TABLE 1. MUSCLE WEIGHTS OF UNTREATED GROUPS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	MEAN WT. OF GASTROCNEMIUS		CORRELATION COEFF.	T RATIO
			Left	Right		
		days	gm.	gm.		
5th.....	21	14	1.154	1.205	.78	1.98
5th.....	16	28	1.101	1.119	.01	.166
4th, 5th.....	26	14	1.120	1.154	.79	1.21
4th, 5th, 6th.....	22	14	.893	.885	.57	.228

TABLE 2. MUSCLE TENSIONS OF UNTREATED GROUPS, IN GRAMS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	DIRECT STIMULATION (THROUGH MUSCLE)				INDIRECT STIMULATION (THROUGH NERVE)			
			Left	Right	Correlation coeff.	T ratio	Left	Right	Correlation coeff.	T ratio
		days								
5th.....	17	14	1141.7	1309.8	.80	2.09	667.4	797.2	.75	2.02
5th.....	14	28	1057.1	1060.7	.24	.018	710.7	675.0	.27	.212
4th, 5th.....	22	14	992.3	1019.3	.63	.502	336.0	304.8	.68	1.58
4th, 5th, 6th.....	21	14	814.2	763.6	.60	2.04				

TABLE 3. MUSCLE WEIGHTS OF TREATED GROUPS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	MEAN WT. OF GASTROCNEMIUS		% DIFFERENCE	T RATIO
			Stimulated muscle	Untreated muscle		
		days	gm.	gm.		
5th.....	53	14	1.204	1.126	6.9	3.25 <sup>1</sup>
5th.....	19	28	1.165	1.021	14.1 <sup>2</sup>	2.57 <sup>2</sup>
4th, 5th.....	24	14	1.157	.977	18.4 <sup>2</sup>	5.81 <sup>1</sup>
4th, 5th, 6th.....	25	14	1.132	.871	29.9 <sup>2</sup>	10.4 <sup>1</sup>

<sup>1</sup> Significant at the 1% level. <sup>2</sup> Significant at the 2% level. <sup>3</sup> These differences are significantly greater than that of the 5th nerve, 14-day group.

TABLE 4. MUSCLE TENSIONS OF TREATED GROUPS, IN GRAMS

SPINAL NERVES CUT	NO. OF RATS	TIME OF DENERVATION	DIRECT STIMULATION (THROUGH MUSCLE)				INDIRECT STIMULATION (THROUGH NERVE)			
			Stimulated muscle	Untreated muscle	% difference	T ratio	Stimulated muscle	Untreated muscle	% difference	T ratio
		days								
5th.....	50	14	1255.1	1165.3	7.7	2.43 <sup>1</sup>	727.3	722.3	0.7	.081
5th.....	19	28	1136.3	847.3	39.5 <sup>2</sup>	3.65 <sup>2</sup>	779.0	712.6	9.3	.758
4th, 5th.....	19	14	874.4	680.3	28.5 <sup>2</sup>	3.09 <sup>2</sup>	210.7	258.3	22.6	1.45
4th, 5th, 6th.....	25	14	1013.7	760.2	33.8 <sup>2</sup>	8.6 <sup>2</sup>				

<sup>1</sup> Significant at 2% level. <sup>2</sup> Significant at 1% level. <sup>3</sup> These differences are significantly greater than that of the 5th nerve, 14-day group.

- b) 16 rats. No treatment. Sacrificed 28 days following nerve sections.
- c) 53 rats. Electrical stimulation of one gastrocnemius. Sacrificed 14 days following nerve section.
- d) 19 rats. Electrical stimulation of one gastrocnemius. Sacrificed 28 days following nerve section.
- Group 2. Severe partial denervation. Bilateral section of fourth and fifth lumbar nerves.
  - a) 26 rats. No treatment. Sacrificed 14 days following nerve section.
  - b) 24 rats. Electrical stimulation of one gastrocnemius. Sacrificed 14 days following nerve section.
- Group 3. Complete denervation. Bilateral section of fourth, fifth and sixth lumbar nerves.
  - a) 22 rats. No treatment. Sacrificed 14 days following nerve section.
  - b) 25 rats. Electrical stimulation of one gastrocnemius. Sacrificed 14 days following nerve section.

Statistical significance was determined by the method of paired comparisons.

### RESULTS

*Weight and Strength of Untreated, Control Groups.* An examination of tables 1 and 2 reveals that there are no significant differences in either wet weights or in muscle tensions through direct and indirect (nerve) stimulation between the left and right gastrocnemii for any of these groups. With the exception of the 28-day group, a fair correlation of weight and strength loss exists between the muscle pairs. Thus, in most instances following bilateral section of corresponding spinal nerves, both gastrocnemii of the rat undergo essentially the same degree of atrophy. The application of an effective therapeutic agent to one of the muscles should then yield statistically significant differences in weight and strength between the muscles.

*Muscle Weight and Strength of Treated Groups.* In all these groups, daily electrical stimulation significantly retarded the loss of weight and strength of the treated muscle as compared to its paired untreated control (tables 3 and 4). These differences although small in the case of the 14-day group with fifth lumbar nerve section become significantly greater as either the denervation is made more complete or its duration prolonged (table 4).

In no case was there any significant difference in muscle tension between treated and untreated muscles when tension development was elicited by indirect (nerve) stimulation. The greater strength and presumably the greater mass of the treated muscles must be due to the effects of electrical exercise upon those contractile units which have lost their innervation. That portion of the muscle with intact innervation is apparently unaffected by the regimen of electrical stimulation.

### DISCUSSION

The results reported here conclusively demonstrate that, in the rat, electrical stimulation significantly retards the atrophy which a muscle undergoes following a partial loss of its motor innervation as well as that resulting from a complete denervation.

It is apparent, however, that the loss of motor innervation must be of considerable severity or duration before the weight and strength difference between treated and untreated muscles are sufficiently great to justify the use of electrical stimulation as a therapeutic agent in paralysis of skeletal muscle.

Thus, the increased weight and strength of the stimulated muscles when only the fifth lumbar nerves are sectioned for a period of 14 days, although statistically significant ( $t = 3.25, 2.43$ ), are not particularly striking. On the other hand, when either the existing denervation is maintained for a longer period (28 days) or the motor innervation is subjected to a still further reduction, considerable and, presumably, important differences occur in the mass and contractile power of the muscles.

Since electrical stimulation does not significantly alter the tension responses of those parts of the muscle which still have a motor nerve supply, it is reasonable to conclude that: 1) the beneficial effects of electrical stimulation result from its action upon the denervated muscle fibers and 2) electrical stimulation, per se, does not have any deleterious effect upon those fibers with an intact innervation.

#### SUMMARY

1. Electrical stimulation by means of a modulated sinusoidal current with a carrier frequency of 25 cycles per second significantly retards the weight and strength loss of the gastrocnemius muscle of the rat which occurs following partial as well as complete motor denervation.

2. The differences in weight and strength between treated and untreated muscles, which have been subjected to a partial denervation, become significantly greater as either the period of denervation or the extent of the denervation is increased.

3. The difference in strength (and presumably weight) between treated and untreated muscles is due to the effect of the electrical stimulation upon those fibers which have lost their innervation.

4. The tension developed by those fibers whose innervation is still intact is apparently unaffected by daily electrical stimulation for periods of 14 to 28 days.

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# DISSOCIATION OF POTASSIUM AND ACETYLCHOLINE SENSITIVITY OF FROG MUSCLE PRODUCED BY ISOTONIC GLUCOSE

E. VANREMOORTERE<sup>1</sup>

*From the Cardiovascular Department, Medical Research Institute, Michael Reese Hospital*

CHICAGO, ILLINOIS

MANY pharmacological agents are known to modify the reaction of striated muscle to potassium and acetylcholine. For example, veratrine and thiocyanate markedly increase the sensitivity of muscular tissue to potassium and increase its sensitivity to acetylcholine to a lesser extent (1-3). Physostigmine, on the other hand potentiates the action of acetylcholine more than that of potassium (4, 6). Some substances may even produce a dissociation in the sensitivity of the muscle, with an increased response to acetylcholine and a decreased response to potassium. These latter cases of complete dissociation are relatively rare and their interpretation is difficult. From the results reported by Torda and Wolff (4-6), it appears that the combination of decreased acetylcholine sensitivity and increased potassium sensitivity is very unusual.

In the course of a study of the 'veratrinic' action of sodium thiocyanate (3), we have observed a striking example of this type of dissociation, which was regularly produced by isotonic glucose solution and seemed worth reporting.

## METHOD

Ten frogs (*Rana pipiens*) were used in this study. After pithing the animal, the rectus abdominis was excised and divided along the median line. Each half-muscle was used in a separate experiment. Many tests were performed on the same preparation.

After excision the muscles were soaked in Ringer's Solution and kept in the refrigerator for one to four hours. The Ringer's Solution used throughout the experiments had the following composition: NaCl 0.6 per cent; KCl 0.0075 per cent; CaCl<sub>2</sub> 0.01 per cent; NaHCO<sub>3</sub> 0.1 per cent; distilled water.

When ready for use, each muscle was suspended in a muscle chamber containing 10 cc. of oxygenated Ringer's Solution at room temperature. The length of the muscle was isotonically recorded on a kymograph by means of a muscle lever and an ink writing pen.

Shortening was induced by injection into the bath of varying amounts of potassium chloride solution (1-2%) or acetylcholine<sup>2</sup> (5-10 mg/100 cc). The muscle was

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Received for publication July 14, 1948.

<sup>1</sup> Fellow of the Belgian-American Educational Foundation. This department is supported in part by the Michael Reese Research Foundation.

<sup>2</sup> Acetylcholine was generously supplied by Hoffmann-La Roche, Inc., Nutley, N. J.



then exposed to an isotonic solution of glucose for 1 to 15 minutes and the same substances were tested again. A third test was performed after the muscle had been washed with Ringer's Solution.

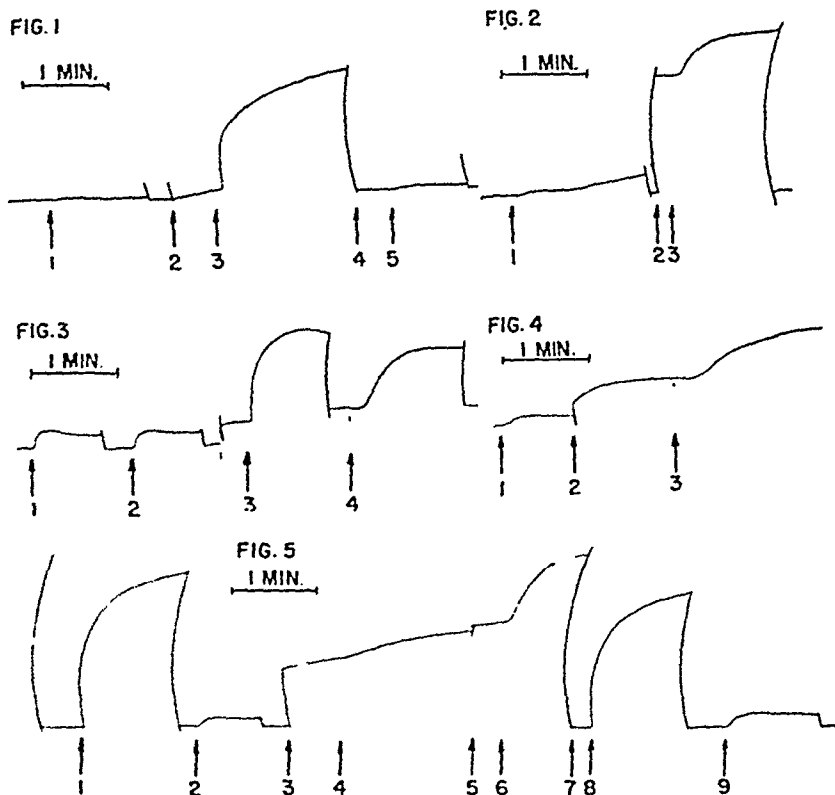


Fig. 1. 1. Injection into the bath of 0.5 cc KCl 2%—muscle in Ringer's Solution. 2. Recording started 30 sec. after replacement of Ringer's Solution by isotonic glucose. 3. 0.5 cc KCl 2%—muscle in glucose. 4. Ringer's Solution for 3 minutes. 5. 0.5 cc KCl 2%—muscle in Ringer's Solution.

Fig. 2. 1. 0.5 cc KCl 2%—muscle in Ringer's Solution. 2. Isotonic glucose in 15 min. 3. 0.5 cc KCl 2%—muscle in glucose.

Fig. 3. 1. 0.5 cc KCl 2%—muscle in Ringer's Solution. 2. 0.5 cc KCl 2%—muscle in Ringer's Solution. 3. 0.5 cc KCl 2%—muscle in glucose for 2 min. 4. 0.5 cc KCl 2%—muscle in glucose for 6 min.

Fig. 4. 1. 0.5 cc KCl 2%—muscle in Ringer's Solution. 2. Isotonic glucose for 3 minutes. 3. 0.5 cc KCl 2%—muscle in glucose.

Fig. 5. 1. 0.25 cc acetylcholine (10 mg/100 cc.)—muscle in Ringer's Solution. 2. 0.5 cc KCl 2%—muscle in Ringer's Solution. 3. Isotonic glucose for 3 min. 4. 0.25 cc acetylcholine—muscle in glucose. 5. Isotonic glucose for 1 min. 6. 0.5 cc KCl 2%—muscle in glucose. 7. Ringer's Solution for 5 minutes. 8. 0.25 cc acetylcholine—muscle in Ringer's Solution. 9. 0.5 cc KCl 2%—muscle in Ringer's Solution.

## RESULTS

A few seconds after the rectus has been in contact with the isotonic glucose, it usually develops a spontaneous shortening which varies in its intensity from muscle to muscle. In most cases the degree of this glucose contracture remains small or moderate and does not disturb the course of the experiment. It soon reaches a steady level or at most increases so slowly that the effect of the substances tested can be evaluated with a fair approximation.

During this sugar contracture the acetylcholine sensitivity is markedly decreased and continues to decrease as long as the muscle remains in contact with the isotonic glucose solution. This rapid loss of acetylcholine sensitivity justified the term: sugar non-irritability (7). The sugar contracture disappears and the action of acetylcholine returns to normal shortly after the solution of glucose is replaced by normal Ringer's Solution. In our experiments the muscle was never allowed to remain in contact with the glucose for more than 15 minutes and the complete reversibility of the phenomenon was always easily demonstrated.

In striking contrast to this effect of isotonic glucose, the potassium sensitivity of the muscle is considerably increased during the sugar contracture. The intensity of the sensitization is comparable to the action of a powerful sensitizer to potassium such as sodium thiocyanate. In spite of the sugar contracture already present in most cases, the effect of the same dose of potassium chloride can be multiplied by five, ten or more times (fig. 1). The shortening induced by potassium under these conditions starts suddenly and rapidly reaches a high level. This effect, however, is not immediate. In most cases the injection is followed by a relatively long latent period before the shortening starts. In some preparations it was possible to record a small but definite relaxation of the muscle following the injection and preceding the potassium-induced shortening which, in these cases, was apparently less marked and less rapid than usual (figs. 3 & 4).

The sensitization to the potassium ion is very marked and perhaps maximal after the muscle has been soaked in isotonic glucose for about one minute (fig. 1). The reaction to potassium seems to decrease as the exposure to glucose is prolonged. Part of this may be an illusion due to the progressive development of the sugar contracture which makes exact quantitative comparisons difficult. After 15 minutes of contact, the effect of potassium is still definitely greater than normal, even when it is superimposed on a marked sugar contracture (fig. 2).

The increased potassium sensitivity observed under these circumstances is also immediately reversed by replacing the potassium with Ringer's Solution (fig. 1).

The opposite changes on potassium and acetylcholine sensitivity of the rectus produced by isotonic glucose are particularly striking when acetylcholine and potassium chloride are tested successively on the same muscle. If the respective concentrations have been chosen in order to produce a much greater initial effect by acetylcholine than by potassium in Ringer's Solution, the ratio is reversed under the influence of the glucose solution and the effect of potassium chloride becomes predominant (fig. 5). The muscle recovers its normal properties very shortly after washing with normal Ringer's Solution.

#### DISCUSSION

The effects of isotonic sugar solution on different frog muscles have been listed by Fenn (7): reversible non-irritability, slight contracture, increase in oxygen consumption, loss of electrolytes by diffusion (chiefly potassium and phosphoric acid), increased lactic acid concentration and an electrical change in the muscle which is temporarily negative but predominantly positive. Previous treatment with sugar prevents the contracture and the rise in oxygen consumption due to potassium (7),

but exposure to glucose was much more prolonged in such experiments than in the present ones in which we were concerned exclusively with the early effects of isotonic glucose.

Our findings confirm Fenn's description of the 'sugar contracture'. They also demonstrate a peculiar dissociation of potassium and acetylcholine sensitivity occurring very shortly after the exposure of the muscle to isotonic glucose. The action of potassium chloride is markedly increased. Some of our results, however, suggest that an antagonism between the action of glucose and potassium can be observed even in this early period; this is apparent in the long latent period following the injection of potassium chloride and especially for the temporary relaxation which is sometimes observed as a first effect of the injection. These are probably transitional phenomena which precede the effects reported by Fenn (7).

The opposite changes in potassium and acetylcholine sensitivity described in this report find no simple explanation. A loss of potassium from muscle decreases its sensitivity to potassium. Lactic acid increases it, but does not affect the acetylcholine sensitivity (4). Calcium-free Ringer's Solution increases the response to potassium but the effect of acetylcholine is decreased significantly only after long exposure (4).

The rôle of the sodium ion is probably predominant: muscles presenting the sugar non-irritability recover when again immersed in Ringer's Solution or any non-toxic solution containing sodium ions (8). Further, we did not observe any increase in potassium sensitivity when isotonic sodium chloride was used instead of isotonic glucose. It is probable that the changes in acetylcholine and potassium sensitivity are related to an ionic disturbance produced by isotonic glucose and involving the sodium ion. Their real mechanism, however, is still undetermined.

#### SUMMARY

Early effects of isotonic glucose on the frog's rectus abdominis include a small or moderate contracture, a decrease of acetylcholine sensitivity and a marked increase in potassium sensitivity. These changes are easily reversible. The increased reaction to potassium is often preceded by a relatively long latent period and sometimes by a slight relaxation of the muscle. The mechanism of these phenomena is not established.

I am greatly indebted to Dr. L. N. Katz for his advice and criticism in the course of these experiments and in the preparation of this report.

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# PLASMA PROTEIN CONCENTRATIONS AND ORGAN WEIGHTS OF CASTRATED AND TESTOSTERONE PROPIONATE TREATED RATS<sup>1</sup>

JAMES H. LEATHEM

*From the Bureau of Biological Research, Rutgers University*

NEW BRUNSWICK, NEW JERSEY

**H**YPOTHYROIDISM induces an increase in plasma globulin concentration in the rat without significantly altering plasma albumin levels. This increase in plasma globulin was observed in adult male rats made hypothyroid by feeding thiourea (1), but at the same time a decrease in seminal vesicle weight was observed thus raising the question as to the part played by androgen in these plasma protein changes. The decrease in seminal vesicle weight could not be correlated with a decrease in food intake or with total gonadotrophic hormone content of the pituitary (2). Smelser (3) previously observed a seminal vesicle weight loss in the absence of a change in pituitary gonadotrophic hormone content in thyroidectomized rats. The effect of thiourea on seminal vesicle weight suggested an androgen deficiency which, in view of the known relationship between androgens and protein metabolism (4), raised a question as to the importance of hypogonadism in plasma protein changes. The clinical studies involving androgens and serum proteins have provided varied results (5-9) and except for the studies of Abels and his co-workers (8) have involved only total serum proteins. In the experiments reported here the plasma globulin concentrations can be seen to be altered by androgen. Since the influence of androgen on organs other than those of the reproductive system is still controversial, the autopsy data is also presented.

## MATERIALS AND METHODS

Male rats of the Long-Evans strain were used when 150 to 155 days of age. The rats were caged in pairs in metabolism cages for measurement of daily food intake. The diet consisted of Purina calf chow plus 10 per cent meat scrap, the meat scrap containing 55 per cent protein. The protein content of this diet was 33 per cent. All animals had been raised on the same diet used during the experimental regime and received supplements consisting of a mixture of two-thirds mazola and one-third cod liver oil on bread twice weekly and of fresh carrots once each week.

Castrated rats were injected on the day following gonad removal. Testosterone propionate (Perandren, Ciba<sup>2</sup>) was administered subcutaneously daily in oil (0.1 cc). After a 20- to 25-day experimental period, the rats were lightly anesthetized with ether and bled from the heart. Hematocrit, non-protein nitrogen, total plasma protein, albumin and globulin were determined by methods

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Received for publication July 15, 1948.

<sup>1</sup> Initially supported by the Protein Metabolism Fund of the Bureau of Biological Research, Rutgers University, and completed under contract with the Office of Naval Research, Navy Department.

<sup>2</sup> Testosterone propionate (Perandren, Ciba) was supplied by Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Summit, N. J.

reported previously (1). Each rat was autopsied and the fresh weight of the pituitary, adrenal, thyroid, seminal vesicles, kidneys, liver and, in some instances, ventral prostate and spleen were recorded.

### RESULTS

Castrated rats were permitted to eat *ad libitum* and food consumption was at a normal level although the castrated rats gained on the average only 5 gm. as compared to a 17-gm. body weight gain by the controls. The castrated rats exhibited total plasma protein concentrations well above normal and due entirely to a rise in plasma globulin (table 1). Plasma albumin, non-protein nitrogen and hematocrit levels were not significantly altered.

Replacement therapy in castrated rats was initially studied at 1.25 mg. daily. Table 1 reveals that the androgen at this level will prevent the rise in plasma globulin

TABLE 1. PLASMA PROTEIN CONCENTRATIONS IN CASTRATED AND TESTOSTERONE PROPIONATE TREATED RATS

NO. OF RATS	TREATMENT	BODY WT., START— END	HEMATOCRIT	NON- PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN
		gm.	%	mg./100 cc.	gm./100 cc.	gm./100 cc.	gm./100 cc.
20	Normal	235-252	45.4 ± 0.7 <sup>2</sup>	54 ± 0.8	6.37 ± 0.07	3.86 ± 0.09	2.51 ± 0.10
20	Castrated	268-273	44.5 ± 0.7	54 ± 2.4	6.65 ± 0.10	3.69 ± 0.10	2.96 ± 0.13
8	Castrated + 0.1 mg. T.P. <sup>1</sup>	270-280	46.6 ± 0.9	66 ± 4.2	6.04 ± 0.13	3.60 ± 0.10	2.44 ± 0.13
10	Castrated + 0.25 mg. T.P.	240-258	46.5 ± 1.0	96 ± 5.0	6.21 ± 0.15	3.76 ± 0.14	2.45 ± 0.11
11	Castrated + 1.25 mg. T.P.	272-262	48.2 ± 0.8	58 ± 2.7	6.44 ± 0.09	3.87 ± 0.09	2.57 ± 0.13
11	Normal + 0.5 mg. T.P.	310-322	47.0 ± 0.7	54 ± 0.9	6.13 ± 0.12	3.62 ± 0.11	2.51 ± 0.15
14	Normal + 1.25 mg. T.P.	259-264	49.0 ± 0.8	61 ± 3.9	6.68 ± 0.12	3.91 ± 0.09	2.77 ± 0.10
8	Normal + 2.5 mg. T.P.	280-279	50.0 ± 0.7	68 ± 3.9	5.92 ± 0.18	3.92 ± 0.31	2.00 ± 0.17

<sup>1</sup> T.P. = Testosterone propionate.  $\sigma_c = \sqrt{\frac{\sum d^2}{N(N-1)}}$

concentration and, in fact, maintains plasma levels at normality. These rats, however, lost on the average of 10 grams of body weight during the experimental period and on autopsy the excessively stimulated accessory sex organs indicated the hormone overdosage (table 2). Resorting to daily dosages of 0.1 mg. and 0.25 mg., it was found that both of these amounts would adequately prevent the rise in plasma globulin concentration while permitting body weight to increase. Using seminal vesicle weight as a criterion, it was apparent that 0.1 mg. of testosterone propionate more closely approximated a physiological dose than the larger amounts used (table 2). For no apparent reason the non-protein nitrogen of castrated rats receiving 0.25 mg. of the androgen was very high (table 1).

TABLE 2. ORGAN WEIGHTS OF CASTRATED AND NORMAL RATS TREATED WITH TESTOSTERONE PROPIONATE

NO. OF RATS	TREATMENT	PITUITARY	ADRENAL	THYROID	TESTIS	SEMINAL VESICLE	VENTRAL PROSTATE	SPLEEN	KIDNEY	LIVER
						<i>mg./100 gm. body wt.</i>				
20	None	3.0 ± 0.3 <sup>1</sup>	10.0 ± 0.2	8.0 ± 0.1	1063 ± 77	377 ± 115	80 ± 7	314 ± 29	919 ± 37	3772 ± 147
20	Castrated	3.4 ± 0.1	9.7 ± 0.4	6.8 ± 0.2		54 ± 2.0	11 ± 0.6	422 ± 33	849 ± 25	3769 ± 138
8	Castrated + 0.1 mg. T.P.	3.5 ± 0.1	10.2 ± 1.0	9.3 ± 0.2		409 ± 19	121 ± 12	609 ± 62	1006 ± 37	4762 ± 151
10	Castrated + 0.25 mg. T.P.	2.8 ± 0.1	10.0 ± 0.5	7.7 ± 0.6		636 ± 30	173 ± 10	516 ± 43	947 ± 41	4459 ± 132
11	Castrated + 1.25 mg. T.P.	2.9 ± 0.1	8.9 ± 0.5	7.3 ± 0.4		962 ± 28	218 ± 10	468 ± 57	989 ± 47	3866 ± 221
11	Normal + 0.5 mg. T.P.	2.5 ± 0.1	7.0 ± 0.5	7.1 ± 0.4	805 ± 48	739 ± 24			844 ± 42	3837 ± 295
14	Normal + 1.25 mg. T.P.	2.8 ± 0.1	8.3 ± 0.4	6.4 ± 0.3	990 ± 37	990 ± 37	250 ± 13	325 ± 15	911 ± 27	3665 ± 132
8	Normal + 2.5 mg. T.P.	2.8 ± 0.1	9.9 ± 0.5	6.8 ± 0.4	969 ± 41	969 ± 41	256 ± 16	339 ± 40	984 ± 42	3634 ± 162

$$1e = \sqrt{\frac{\sum d^2}{N(N-1)}}$$

The effect of testosterone propionate on the plasma protein concentrations of normal rats was studied following administration of daily dosages of 0.5 mg., 1.25 mg. and 2.5 mg. The smaller dosage permitted normal body weight increases but the larger doses suppressed the growth rate. The 1.25 mg. and 2.5 mg. amounts of androgen had a tendency to increase hematocrit and non-protein nitrogen whereas the effect on plasma protein concentrations was varied (table 1). Unlike the castrated rat, a daily dosage of 0.5 mg. of androgen failed to alter the plasma protein concentrations in the normal rat. The 1.25 mg. dosage was followed by a tendency for total protein levels to be increased, due to an increase in globulin, but the 2.5 mg. dosage exercised quite the opposite effect on the globulin levels. This latter dosage provided data that were widely variable.

After castration, the reproductive organs were atrophic but significant weight changes were not recorded for the pituitary, adrenals or liver. Kidney and thyroid weights had decreased somewhat whereas splenic weight was increased (table 2). Testosterone propionate at 0.1 mg. daily readily maintained the accessory reproductive organs but larger doses induced excessive stimulation. Any tendency toward pituitary hypertrophy was abolished by the androgen but a weight increase of the spleen, kidney and liver, over that of the normal as well as the castrated rat, was obtained with 0.1 mg. daily. Increased dosages were less effective so that administration of as much as 1.25 mg. daily abolished the liver hypertrophy and retained the kidney weight at normalcy. Spleens of the androgen treated castrates were heavier in each group but did not exceed castrate level when a 1.25 mg. dosage was used. In normal rats, the androgen exerted the anticipated effects on the reproductive system but, despite the protein anabolic action assigned to testosterone, it failed to increase kidney, liver or splenic weights in relationship to body weight and body weight gain was not accentuated. Thyroid weight and in some cases adrenal weight was decreased by androgen (table 2).

#### DISCUSSION

Castration induced a significant rise in plasma globulin in 20 days in adult rats. Since a slight but not significant fall in plasma albumin was usually obtained the resultant change in total plasma protein was frequently not striking. One might consider this plasma change as an indirect effect via the thyroid since the BMR of a castrated rat is below normal (10). However, an increase in NPN and a decrease in hematocrit as typical changes in hypothyroidism (1) did not occur after castration. The adrenal cortex may be involved (11) although in this regard adrenal hypertrophy did not occur in our animals and Smith (12) has observed increased adrenal activity only after 56 days of gonadectomy. Fasting will excite the adrenal cortex and may lower the BMR, but this does not seem capable of explaining the changes observed since as much as a 50 per cent reduction in food intake will rarely increase the plasma globulin level significantly.

Although many studies have demonstrated the nitrogen-retaining action of testosterone propionate in castrated animals these studies have not involved the plasma. In the canuroid, androgen administration failed to alter plasma protein levels (5), but in the castrated rat the hormone prevented the rise in plasma globulin following

castration only. Examination of total protein levels would not clearly reveal this action since the slight decrease in albumin would mask the effect. This corrective action of testosterone propionate may or may not be assigned to a thyroid-like stimulation since Kenyon (13) noted an increased BMR in androgen-treated eunuchoids but negative effects on the BMR of the castrated rat have also been reported (10, 14). Testosterone is effective in causing nitrogen retention in normal dogs and rats (15, 16) but plasma protein levels were not reported. Various clinical reports have related the plasma protein concentrations to androgen administration but the results range from a slight increase (7), to no change (9), to a decrease (6, 8). The current studies on normal rats indicate little effect with 0.5 mg. daily, a plasma protein increase with 1.25 mg. and a definite decrease due to a drop in plasma globulin concentration with 2.5 mg. These results only emphasize the need for further study and possibly why the clinical data are conflicting.

Castration has been reported to cause a decrease in liver weight with an increase to greater than normal with testosterone (17) whereas in the normal rat a decrease and in puppies an increase in liver weight followed androgen treatment (18, 19). Castration for 25 days failed to significantly alter liver weight but an increase to above normal was obtained with relatively low dosages of androgen whereas the effect was abolished with larger doses. No effect on liver weight in normal rats was recorded.

Whether or not androgens effect kidney size in the rat is as yet unanswered (4). In our studies, kidney weight/body weight was not influenced in normal rats by 0.5 to 2.5 mg. daily, but restoration of the slight kidney weight loss following castration was readily attained with testosterone propionate.

Castration led to some increase in splenic weight which was augmented by small dosages of androgen. This reaction of the spleen is unlike that of the thymus which decreases after hormone administration (20). No effect on splenic weight was obtained in normal rats with androgen when considered in relation to body weight.

Korenchevsky (21) reported a decrease in thyroid weight to body weight ratio after castration and our results suggest a similar trend. These data have been contested on the basis of actual weights and indeed our data would fail to show any thyroid effect of castration alone if actual weights were compared. Androgen administration to castrated rats keeps thyroid weight normal whereas large doses depress thyroid weight. An increase in thyroid weight in mice and an increase in mitotic figures in rats has been correlated with androgen treatment by other investigators (22, 23).

#### SUMMARY

Castration for 20 to 25 days increased total plasma protein concentrations in adult rats due to an increase in plasma globulin. Plasma albumin had a tendency to be reduced, but not significantly and non-protein nitrogen and hematocrit remained unchanged. Testosterone propionate prevented the plasma protein changes induced by castration but induced varied results in normal male rats.

Three weeks after castration, the reproductive system was atrophic but significant weight changes on a body weight basis were not recorded for the pituitary, ad-



renals or liver. Kidney and thyroid weights had decreased whereas splenic weight was increased. Small doses of testosterone propionate increased the weight of the spleen, liver and kidney in the castrated rat but larger doses were less effective.

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# MECHANISMS OF DESOXYCORTICOSTERONE ACTION. II. RELATION OF SODIUM CHLORIDE INTAKE TO FLUID EXCHANGE, PRESSOR EFFECTS AND SURVIVAL

D. M. GREEN, D. H. COLEMAN AND M. McCABE

*From the Department of Medicine, University of Washington School of Medicine*

SEATTLE, WASHINGTON

THE rôle of sodium as an accessory factor in hypertension and nephrosclerosis has been emphasized during the past half century by an increasing accumulation of human and animal data (1). Inasmuch as sodium metabolism is regulated primarily by the corticosteroids, the possibility exists that the aggravation of the hypertensive states by salt results from their underlying etiologic basis in adrenal cortical dysfunction.

The hypertensive capacity of the corticosteroids is evident in the blood pressure elevations which attend cortical tumors. Its independence of sensitizing procedures as necessary adjuncts has been demonstrated (2) and confirmed experimentally (3) by investigations in which implantation of DCA pellets was followed by the development of hypertension in normal animals to which no supplementary sodium was administered.

These observations have prompted further study to determine first, if maximal increases in salt exchange could provoke hypertension in the absence of adrenocortical dysfunction and second, if the degree of hypertension induced by excessive salt-retaining hormone could be correlated with an increased level of salt intake.

## EXPERIMENTAL PROCEDURES AND RESULTS

*Effects of Sodium Chloride on Weight, Fluid Intake and Blood Pressure.* The first experiment of this section consisted in the substitution of isotonic (0.86%) sodium chloride for drinking water in a group of 24 rats of the Sprague-Dawley strain from the time of weaning until maturity. The group was divided evenly as to sex. A control group of similar composition was maintained on water. The animals were kept in individual cages and fed the drinking fluid and Purina laboratory chow *ad libitum*. Fluid intake and weight were measured on a schedule previously described (4). The data were grouped according to 20-gm. weight intervals.

The results (fig. 1) indicated that unit fluid intake (cc/gm/day) was related inversely to weight in both groups. The intake curves for female animals closely approximated those of males in the same group, despite differences in rate of growth. These relationships in growing animals of the same species were analogous to the results obtained comparing mature animals of differing species, in which fluid exchange was found related to body weight by a fractional exponent (5).

The animals offered saline drank more than the controls throughout the weight

ranges studied. Calculations of the ratio of average saline intake to average water intake at corresponding weights (fig. 1), indicated that the ratio of these intakes remained fixed despite the steady decline in unit intake which occurred as the animals matured.

In the next experiment, groups of 12 male rats were maintained on water, isotonic, twice, and two and one-half times isotonic saline respectively for a period of 14 weeks. The hypertonic solutions were given in order to increase not only the overall salt exchange but the osmotic work of the kidney as well. In addition to the measurements previously described, the blood pressure of each animal was estimated weekly by the tail method (6) from the eighth week onward. All animals were

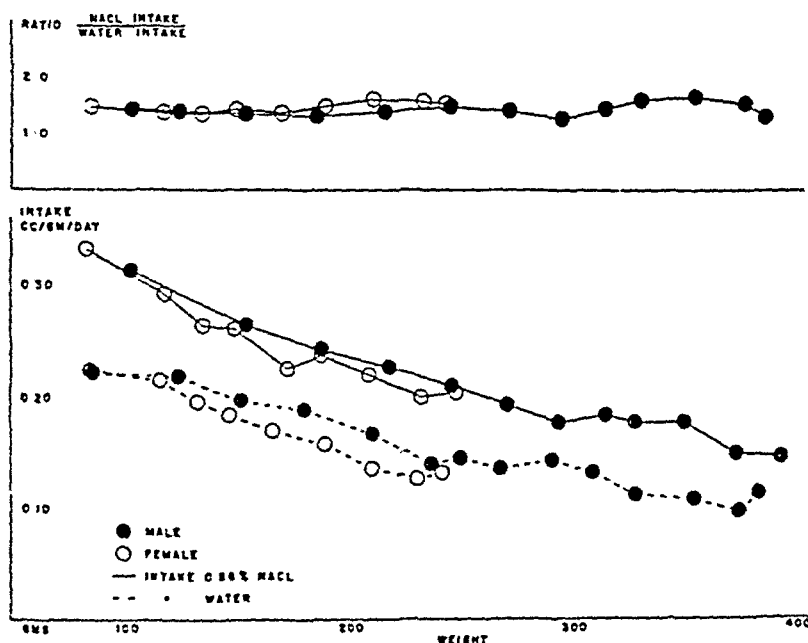


Fig. 1. RELATIONSHIP OF WATER AND ISOTONIC SALINE INTAKES in rats as modified by growth and sex.

autopsied. Analyses of wet and dry tissue weights were made on representative organs of surviving animals in each group.

The growth curves of animals which drank isotonic saline did not differ significantly from those of controls (fig. 2). Fluid intake-weight relationships were as previously described.

The animals given twice isotonic saline showed an initial loss of weight and an increase in intake greater than that exhibited by the previous group. Three of the animals refused to drink after varying periods of time, lost further weight and died. Survival on the contrary, as in animals forced to drink sea water (7), was characterized by a progressive rise in fluid intake to levels three times greater than the control value, accompanied by stabilization of weight or a small gain. As a consequence, the average weight of the group at the conclusion of the test period approximated its initial value.

The animals maintained on two and one-half times isotonic saline presented an exaggerated picture of the preceding group. Loss of weight was progressive through-

out the greater part of the test period. As before, the animals survived if they increased their intake and died when they reduced it. Six of the group completed the entire period. Toward the end their weights had stabilized at values below the initial levels and daily fluid intake approached body weight. The animals spent most of their waking hours drinking.

Despite these marked alterations in growth, weight, water and salt exchange, none of the test groups manifested a rise in blood pressure above control levels. There were no evidences of salt or water loss by vomiting or diarrhea.

*Effect of Sodium Chloride on Organ Weight and Hydration.* Comparison of the percentage organ weights of the test animals with those of water-fed controls, both

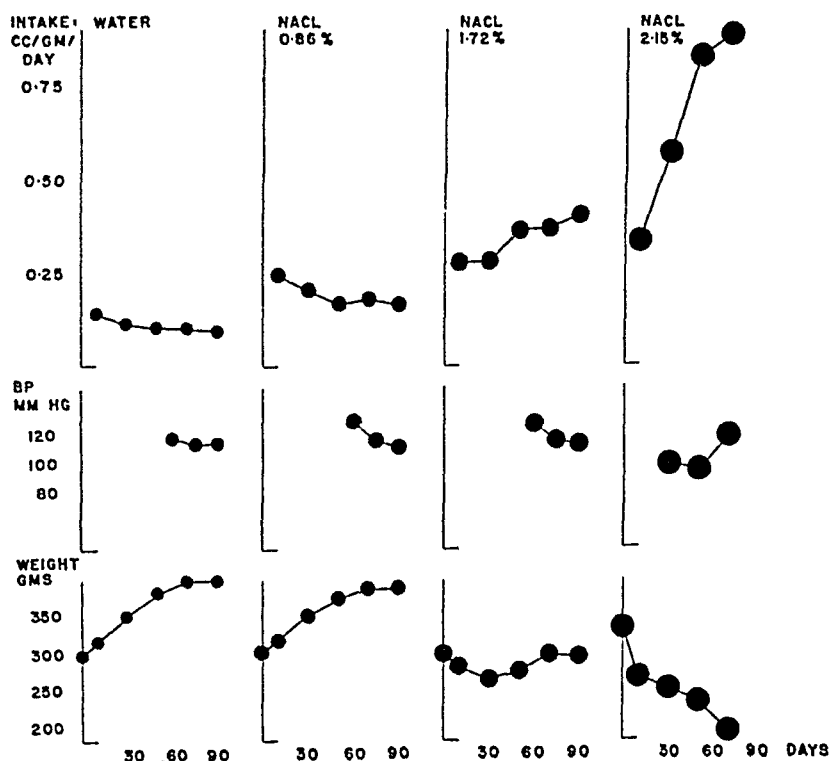


Fig. 2. FLUID INTAKE, BLOOD PRESSURE AND GROWTH of rats in relation to the salt content of the drinking fluid.

normal and starved (table 1), revealed increases in heart and kidney weight in proportion to the concentration of salt in the drinking fluid. These changes appeared too great to be explained entirely on the basis of reduced nutrition. Calculation of wet and dry weight ratios (table 2) indicated the net tissue water content of the test groups to approximate that of the controls.

The results suggest that increased velocity of fluid exchange facilitates the renal excretion of sodium, even when increased velocity is obtainable only at the expense of drinking still further quantities of a salt-containing solution. The effectiveness of the mechanism is evidenced by the maintenance of tissue hydration and prolongation of life in the animals which employed it. It is significant that a general rise in blood pressure was not invoked as a means of increasing glomerular filtration

pressure and consequent sodium clearance, even at levels of salt intake which reached 2 per cent of the body weight per day.

*Effects of DCA on Intake.* Four groups of 10 freshly weaned rats were used in the first experiment of this section. Males and females were represented equally in each group. Two of the four groups were controls, maintained on water and on 0.86 per cent sodium chloride solution respectively. The two test groups were im-

TABLE 1. PERCENTAGE ORGAN WEIGHTS OF RATS FED VARYING SALT CONCENTRATIONS IN DRINKING FLUID

	DRINKING FLUID				
	Water	0.86% NaCl	1.72% NaCl	2.15% NaCl	Water, no food
No. of animals.....	10	12	12	10	7
Body wt. (gm.).....	413	392	279	173	156
Organ (% wt.):					
Adrenals.....	0.015	0.016	0.018	0.040	0.038
Heart.....	0.37	0.41	0.51	0.68	0.53
Kidneys.....	0.84	0.92	1.2	1.3	1.1
Liver.....	4.2	4.3	4.9	4.8	2.4
Spleen.....	0.16	0.17	0.19	0.11	0.21
Testes.....	0.74	0.91	0.68	0.38	1.10

TABLE 2. PERCENTAGE WATER CONTENT IN TISSUES OF RATS FED VARYING SALT CONCENTRATIONS IN DRINKING FLUID

ORGAN	DRINKING FLUID			
	Water	NaCl 0.86%	NaCl 1.72%	NaCl 2.15%
Brain.....	76.7	78.5	79.1	78.5
Heart.....	74.8	78.8	81.6	82.0
Kidney.....	76.8	75.3	80.7	66.2
Liver.....	69.9	73.0	72.8	69.4
Muscle.....	75.2	74.6	77.2	75.1
Skin.....	63.8	62.8	63.3	69.5
Spleen.....	80.6	78.4	81.7	70.1
Average.....	74.0	74.5	76.6	73.0

planted with single 20-mg. DCA pellets after an observation period of one week. One of the test groups was maintained on water and the other on isotonic saline. Weight and fluid intake were measured continuously for a three-month period. The data were grouped for each 20-gm. weight interval.

Immediately following implantation, the level of intake rose in both test groups and reached a peak within 10 days (fig. 3). The test animals maintained on salt solution manifested a maximum rise over the corresponding control level about four times greater than that of the group maintained on water at the same drug dosage. Subsequently, the unit intakes declined but at rates greater than those displayed by

the controls. As a consequence, the intake curves of all four groups tended toward a common value with the passage of time.

The effect of dosage variation on intake was studied in a group of 12 rats maintained on saline. Six animals were implanted with single 20-mg. pellets and the remainder with ten 10-mg. pellets. Comparison of the intake curves with that of a control group (fig. 4) indicated the response to be qualitatively similar at both dosages. The magnitude of the initial rise in intake and the rate at which the intake curve returned toward the control value were greater at the higher dosage.

The sum of these results suggested that the effect of DCA on fluid exchange under various conditions of dosage and drinking fluid was the displacement of the intake curve upward from the control level. Calculations, therefore, were made of

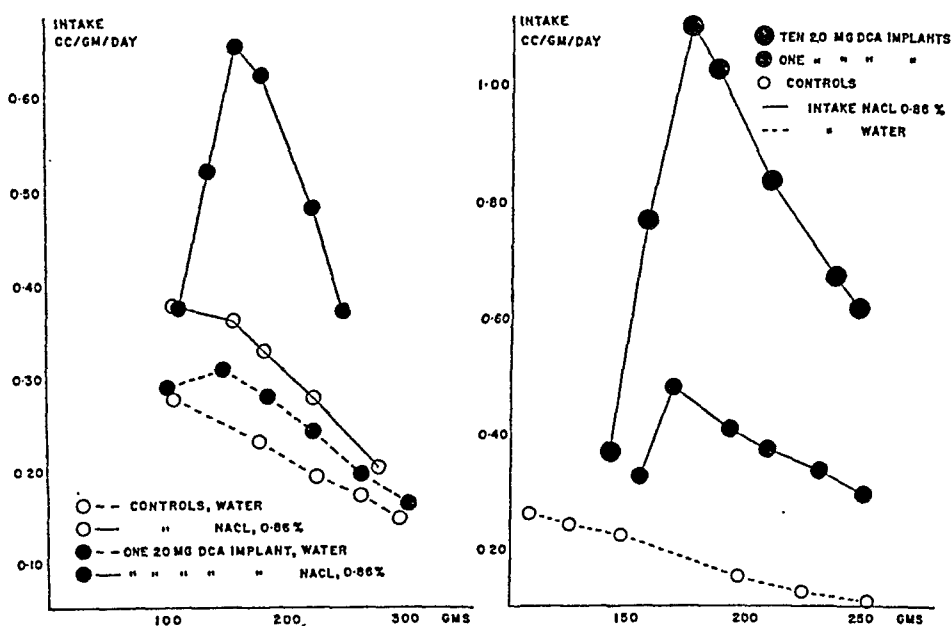


Fig. 3 (left). EFFECT OF DCA IMPLANTS on the intake of rats in relation to the composition of the drinking fluid.

Fig. 4 (right). EFFECT OF DCA IMPLANTS on the intake of rats in relation to dosage.

the ratios of the intake of each test group to the control intake at corresponding weight levels, commencing at the time of attainment of maximum increase in intake. Graphic representation of these ratios (fig. 5) indicated that the basic action of DCA under all circumstances studied was to increase the intake by a factor, the numerical value of which was fixed by the drug dosage and the composition of the drinking fluid. Once established, this ratio tended to remain more or less constant, with some overall tendency to decrease with the passage of time. The data at hand are not sufficient to determine whether this tendency represented a change in the response of the animal to the influence of a fixed dosage of drug, or a slow decrease in dosage due to gradual pellet absorption.

It would appear from these results that the secondary regression of intake levels which follows the initial DCA-induced elevation reflects mainly the magnification of a series of successively decreasing control values by a constant factor.

Further inspection of the displacements in intake curves produced under various conditions indicated that the combined effect of DCA implantation and isotonic saline administration was greater than either the sum or the product of the two separate effects. Thus, the factor of increase, which resulted from implantation of a single 20-mg. DCA pellet in animals also given saline to drink, was found equal to the product of the DCA factor alone multiplied by the square of the salt factor alone, when the numerical values of these factors were determined in groups of animals studied simultaneously. A comparison of the actual changes in unit intake which followed a combination of DCA implantation and isotonic saline administration, with the theoretical curve calculated on the above basis, showed a considerable degree of correspondence (fig. 6).

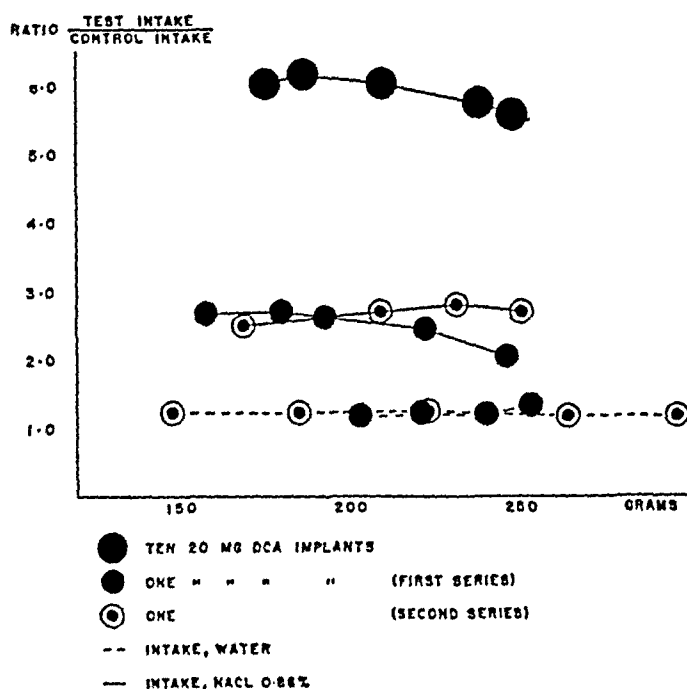


Fig. 5. RATIO OF TEST TO CONTROL INTAKE following DCA implantations as modified by drug dosage and composition of the drinking fluid.

Calculations based on a small number of data derived from animals in which ten 20-mg. pellets had been implanted indicated that the combined factor of intake increase at this higher dosage level approximated the product of the DCA factor multiplied by the cube of the salt factor.

*Effects of DCA on Blood Pressure.* Investigations previously reported (4) have shown that hypertension develops slowly after DCA implantation, being preceded in time by the rise in fluid intake to its peak value. It has been indicated also that salt supplementation is not required to produce blood pressure elevation (2).

These studies have been extended to a total of 113 animals. Recapitulation of the blood pressure changes (table 3) showed that the average maximum pressure of 47 control animals did not exceed 132 mm. Hg. Determinations made on 42 animals implanted with single 20-mg. DCA pellets revealed an average maximum blood

pressure of 158 mm. in those maintained on water as compared with a pressure of 176 mm. in the animals which drank isotonic saline.

In contrast to these differences displayed by water and salt-treated animals at low DCA dosages are the results in animals implanted with ten 20-mg. pellets. Twelve water-fed animals so treated averaged a maximum blood pressure of 209 mm. as compared with a value of 207 mm. in 12 similarly implanted but maintained on isotonic sodium chloride.

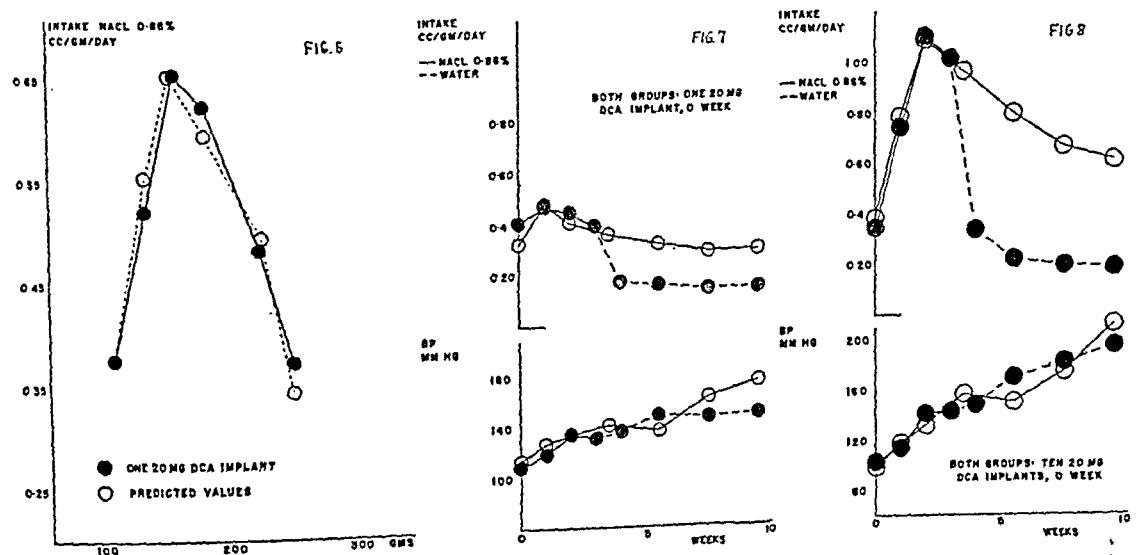


Fig. 6. COMPARISON OF OBSERVED INTAKE CURVE following DCA implantation in rats maintained on isotonic saline with the theoretical intake calculated from the changes produced by DCA and by isotonic saline administration separately.

Fig. 7. EFFECTS OF LOW DCA DOSAGES on fluid intake and blood pressure as modified by the substitution of water for isotonic saline drinking fluid.

Fig. 8. EFFECTS OF HIGH DCA DOSAGES on fluid intake and blood pressure as modified by the substitution of water for isotonic saline drinking fluid.

TABLE 3. AVERAGE MAXIMUM BLOOD PRESSURE OF RATS FOLLOWING DCA IMPLANTATION

DRINKING FLUID	CONTROLS		ONE 20-MG. DCA PELLET		TEN 20-MG. DCA PELLETS	
	No.	BP	No.	BP	No.	BP
Water.....	19	124	20	158	12	209
NaCl 0.86%.....	28	132	22	176	12	207
Average.....	47	128	42	167	24	208

In one experiment of this series 12 animals were implanted with single 20-mg DCA pellets and maintained on isotonic saline until the peak increase in intake had been attained, a period of approximately two weeks (fig. 7). At this time half of the animals were given water to drink while the remainder were continued on saline solution. The fluid intake of the animals placed on water immediately dropped to levels similar to those previously described for implanted animals maintained on water from the outset. However, the blood pressure of these animals continued a



slow progression to a maximum level of 149 mm. Hg, as compared with 174 mm. Hg in the animals maintained on saline throughout the test period. This experiment was repeated with 12 additional animals, in which ten 20-mg. pellets were implanted (fig. 8). The substitution of water for saline produced effects similar to those observed at the lower DCA dosage, but the blood pressure differences were not as great.

From these results it would appear that the administration of isotonic sodium chloride in place of drinking water increases the degree of hypertension developed by animals implanted with DCA, but that this potentiation is less marked when the DCA dosage is increased.

*Effects of DCA on Organ Weight and Hydration.* The percentage weights of representative organs from 56 DCA-treated rats were compared with those of 17 controls (table 4). The treated animals had been implanted for 10 or more weeks before spontaneous death or termination. The average body weight of test animals

TABLE 4. PERCENTAGE ORGAN WEIGHTS OF RATS IMPLANTED WITH DCA AT TWO DOSAGE LEVELS

	CONTROLS	ONE 20-MG. DCA IMPLANT	TEN 20-MG. DCA IMPLANTS		
	Drinking fluid				
	Water	Water	NaCl 0.86%	Water	NaCl 0.86%
No. of animals.....	17	12	18	12	12
Body wt. (gm.).....	309	292	269	295	238
Organ (% wt.):					
Adrenals.....	0.021	0.019	0.024	0.025	0.028
Heart.....	0.39	0.46	0.53	0.55	0.67
Kidneys.....	0.95	1.1	1.2	1.2	1.7
Liver.....	4.8	4.8	4.7	5.3	5.6
Spleen.....	0.20	0.25	0.28	0.25	0.56
Testes.....	0.77	0.98	0.91	0.94	0.95

maintained on water differed little from that of controls. The earlier death of many saline-supplemented animals tended to lower the average weight in this group.

The heart and kidneys were enlarged in all test groups. Salt supplementation augmented this increase at both dosage levels. It appeared notable that the size of these two organs was as great in DCA-treated animals maintained on water as in animals which drank hypertonic saline in the absence of DCA.

The spleen and testes of the DCA-treated animals, unlike those of animals maintained solely on hypertonic saline, were perceptibly heavier than those of controls. At the higher DCA dosage level, the liver also was enlarged, a finding which may have significance in view of the rôle of the liver in DCA detoxification (8). In contrast to determinations made under other experimental conditions (9) adrenal size also tended to be increased.

Calculation of wet and dry weight ratios of representative tissues of test animals demonstrated a tendency to increased water content, more evident in the animals maintained on saline. The increase did not appear sufficient to explain the higher organ weights on the basis of edema.

*Effects of DCA on Survival.* A group of 39 rats was observed for 22 weeks following implantation of single 20-mg. DCA pellets. Autopsy findings showed pellet absorption to be greater than 95 per cent complete at the end of this period. Calculations of survival times (table 6) indicated that only 2 of the 19 animals maintained on water had died during this period as compared with 10 of the 22 animals given isotonic saline. Since, as previously described, the maximum pressures developed at this dosage are not much higher in the salt-supplemented animals, it would appear that the decrease in life expectancy produced by extra sodium ion in DCA-treated animals is disproportionate to its effect on blood pressure.

TABLE 5. PERCENTAGE WATER CONTENT IN TISSUES OF RATS IMPLANTED WITH DCA AT TWO DOSAGE LEVELS

	DOSE LEVELS					
	CONTROLS		ONE 20-MG. DCA IMPLANT		TEN 20-MG. DCA IMPLANTS*	
	Drinking fluid					
	Water	NaCl 0.86%	Water	NaCl 0.86%	Water	NaCl 0.86%
No. of animals.....	3	3	4	4	4	3
Organ:						
Brain.....	76.7	78.5	78.8	78.7	74.0	80.9
Heart.....	74.8	78.8	78.4	80.5	78.6	81.2
Kidney.....	76.8	75.3	75.2	78.6	77.6	79.2
Liver.....	69.9	73.0	69.5	72.8	72.1	73.1
Muscle.....	75.2	74.6	77.4	75.0	78.9	78.1
Skin.....	63.8	62.8	64.1	70.7	69.0	69.5
Spleen.....	80.6	78.4	80.4	75.0	79.2	80.6
Average.....	74.0	74.5	74.8	75.9	75.6	77.5

TABLE 6. AVERAGE SURVIVAL OF RATS IMPLANTED WITH ONE 20-MG. DCA PELLET. (PERIOD OF OBSERVATION: 159 DAYS)

DRINKING FLUID	NO. OF ANIMALS	SURVIVAL days
Water.....	17	150
NaCl 0.86%.....	22	125

The survival period of the 12 animals which did not live out the 22-week period averaged 83 days. This time corresponded closely with the interval of maximum blood pressure increase as determined by serial measurements. It would seem, therefore, that DCA hypertension and its anatomical sequelae do not represent an irreversible threat to the life of the animal, but are lethal only during the period of maximum drug action. These results suggest that if human hypertension is sustained by a mechanism involving increased cortical activity, cure may follow the removal of the offending instrument or the nullification of its activity.

#### SUMMARY AND CONCLUSIONS

Increased sodium intake in rats up to two per cent of body weight per day was accompanied by elevation of fluid exchange, increased heart and kidney weight and

reduction in growth rate, but did not provoke hypertension. The data indicate that increased velocity of fluid exchange represents a mechanism for augmenting the renal excretion of sodium, even under circumstances in which the increase in velocity entails further intake of a salt-containing solution.

The primary effect of DCA was to elevate the level of fluid exchange over control values by a ratio fixed by the dose of the drug and the amount of supplementary salt administration. Hypertension was a subsequent development. The magnitude of DCA-induced hypertension did not correlate with the level of salt exchange, although it was augmented by salt administration, particularly at low DCA dosage levels. The effects of salt supplementation of DCA-implants on heart and kidney weight and on survival were disproportionate to the measured augmentation of blood pressure elevation. The resemblance between the action of salt in the DCA-induced hypertension of animals to that in essential hypertension lends additional support to the hypothesis that the mechanism of sustained pressure elevation in the human involves increased activity of the adrenal cortex. The favorable survival time of DCA-treated animals, once the period of most intense drug action had been passed, suggests the possibility of arresting human hypertensive disease either by removal of the adrenal cortex or by nullification of the activity of its salt-retaining steroids.

We are indebted to Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, N. J., both for generosity in supplying desoxycorticosterone and for many helpful suggestions and criticisms throughout these studies.

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# COMPARISON OF CYCLOPROPANE AND ETHER ANESTHESIA ON LYMPH PRODUCTION

HENRY K. BEECHER, MADELEINE F. WARREN AND ANNA MURPHY

*From the Anesthesia Laboratory of the Harvard Medical School, at the Massachusetts General Hospital*

BOSTON, MASSACHUSETTS

CYCLOPROPANE anesthesia is accompanied by several undesirable effects. In some cases these are of serious nature and uncertain origin (for example, dangerous cardiac irritability). Until these things are explained it will always be of interest to uncover differences in physiological action between cyclopropane and the other common anesthetic agents.

The purpose of this report is to describe a difference in lymph production found under the two anesthetic agents, cyclopropane and ether.

## METHODS

*Animals.* This study is based upon observations made in 11 mongrel dogs. They were allowed water but were fasted for 18 hours before the experiment. Immediately preceding the experiment half of the animals were given 20 cc/kg. of physiological saline over a period of an hour. This made no appreciable difference in the results and will not be discussed further. Blood pressure was determined by the usual direct method. Mean arterial blood pressure, pulse and respiratory rates were recorded at 20-minute intervals. They were comparable for the two agents. Rectal temperatures were observed at half-hour intervals. Two animals were eliminated because of high body temperature. When the subjects are breathing through a 'to-and-fro' soda lime canister in a closed system it is especially important to watch the body temperature of the animals, for it often tends to rise unless the soda lime canister is cooled.

*Anesthesia.* Cyclopropane anesthesia was induced (25% cyclopropane, 75% oxygen) through a plaster cone fitted with a rubber diaphragm. The dog's nose was inserted through this. The hair of its head had previously been wet in order to reduce the danger of electrostatic sparks. Ether anesthesia was induced by the open drop system. In the case of both agents, as soon as the surgical level of anesthesia had been attained, the trachea was cannulated and connected to a closed anesthesia system with to-and-fro breathing through a soda lime canister. Dead space was kept as near normal as possible. During maintenance of anesthesia 85 to 95 per cent oxygen was administered with each agent. Sufficient anesthetic was added from time to time to maintain the same level with both agents, indicated by a sluggish corneal reflex of anesthesia. It is of course important to have a comparable level of anesthesia in all cases.

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Received for publication July 8, 1948.

Shift from one agent to another was easy in the case of cyclopropane to ether, since the former agent is almost entirely eliminated in a few minutes of expiration into the room (ether being inspired at the same time). The reverse shift, ether to cyclopropane, was much more difficult. In this case the anesthesia apparatus was disconnected at the tracheal cannula and the animal allowed to breathe into the air. At the same time a fine rubber catheter was inserted into the trachea, through the cannula, while the dog breathed into the room and pure cyclopropane was administered through the catheter in amounts adequate to keep the dog quietly anesthetized. After about 45 minutes the ether content of the body was greatly reduced. Although some odor of ether always persisted, the concentration was far below that required

TABLE 1. CYCLOPROPANE ANESTHESIA FOLLOWED BY ETHER

NO.	WEIGHT	SEX	CYCLOPROPANE						ETHER					
			Lymph		Blood			Resp/ min.	Lymph		Blood			Resp/ min.
			Flow	Protein	Hema- tocrit	Protein	Mean arter- ial pres- sure		Flow	Protein	Hema- tocrit	Protein	Mean arterial pressure	
			mg./ min.	gm. %	%	gm. %	mm. Hg		mg./ min.	gm. %	%	gm. %	mm. Hg	
1	13.6	♀	84	2.82	56.3	6.12	150	30	144 <sup>1</sup>	1.98	61.4	6.77	125	39
2	11.0	♂	108	1.53	47.0	6.01	152	60	161	1.54	51.1	6.16	118	71
4 <sup>2</sup>	12.2	♀	100	2.20	49.5	7.05	153	80						
5	12.5	♀	74	3.71	48.0	7.02	118	78	112	3.38	51.2	7.12	108	52
11	12.5	♂	88	2.35	54.2	6.94	165	59	140	2.47	59.0	7.09	140	62
12	10.5	♂	56	2.05	45.8	7.65	132	100	140	2.65	45.0	7.87	135	90
Means with			85	2.59	50.1	6.80	145	68	139	2.40	53.6	7.00	125	63
S. E.			±7.6	±0.30	±1.7	±0.25	±6.9	±9.8	±7.9	±0.31	±3.0	±0.28	±5.7	±8.6

<sup>1</sup> One side doubled. <sup>2</sup> The last half of this experiment was interrupted by a technical accident.

for anesthesia. The trachea was then reconnected to the closed anesthesia apparatus and a mixture of cyclopropane and oxygen administered as previously described.

**Lymph Collection.** Lymph was obtained from the cervical lymphatics by the method of McCarrell (1), in which a motor is used to nod the head at the rate of 16 times per minute. Exact reproducibility of the motion of the head of a given dog from one period to another is necessary for quantitative comparison of lymph flow from one collecting period to another. Both main cervical lymphatics were cannulated. In case these vessels were double on a side, often the case, one was tied off. A few particles of heparin were introduced into each cannula and the lymph was collected regularly at five-minute intervals. This was placed in weighed tubes, stoppered, the weight of the lymph determined, and the flow expressed in milligrams per minute. Averages were based upon 45-minute collection periods. Recorded collections were not made until the lymph flow had settled down to a uniform rate, after the start of the experiment or after shifting from one agent to another. This required usually 10 to 15 minutes.

The protein content of the lymph and the plasma (venous blood) were determined refractometrically. The hematocrit was determined in venous blood. The blood samples were drawn about 45 minutes after induction of the respective anesthetics.

## RESULTS

The results are shown in tables 1 and 2. There is wide variation in the lymph flow from one dog to another under a given agent. The variation in lymph production from dog to dog tends to hide the ether or cyclopropane effect in the 'average' animal. This variation is in accord with our previous experience (2). An important point is the fact that each single experiment shows a difference in the same direction: the lymph flow under cyclopropane is always less than it is under ether regardless of which agent is administered first. In absolute terms, the magnitude of these changes is not trivial.

TABLE 2. ETHER ANESTHESIA FOLLOWED BY CYCLOPROPANE

NO.	WEIGHT	SEX	ETHER							CYCLOPROPANE						
			Lymph		Blood			RESP/ MIN.	Lymph		Blood			RESP/ MIN.		
					Hema- tocrit	Protein	Mean Arterial Pressure				Hema- tocrit	Protein	Mean Arterial Pressure			
			Flow	Protein					Flow	Protein					Flow	Protein
	kg.		mg/min	gm. %	%	gm. %	mm. Hg		mg/min.	gm. %	%	gm. %	mm. Hg			
3	12.3	♀	152	3.08	58.5	6.25	98	30	91 <sup>1</sup>	2.45	60.6	6.89	103	50		
7	12.7	♀	63	2.57	56.2	6.63	102	44	45	2.79	54.0	6.23	93	38		
9	9.0	♀	111	2.24	57.0	7.63	118	68	85	2.15	61.0	7.98	130	59		
10	12.5	♀	136	3.03	58.0	7.33	110	63	69	2.93	53.3	7.07	117	70		
13	10.5	♂	77	2.60	56.5	7.24	102	58	62	2.45	57.5	7.70	126	59		
Means with			108	2.70	57.2	7.02	106	53	70	2.55	57.3	7.17	114	55		
S. E.			±16.9	±0.16	±0.4	±0.25	±3.6	±6.9	±8.2	±0.44	±1.6	±0.31	±7	±5.3		

<sup>1</sup> Collection started only 20 min. after last ether period.

The experiments shown here represent consecutive cases, with two exceptions: *Experiments 6 and 8* were not included for the reason that these dogs were found to have high temperatures, 41.1° C and 42.0° C., respectively, early in the procedure.

No striking protein or hematocrit differences were observed.

## DISCUSSION

Probably the correct explanation for the difference in lymph flow observed here is that the capillary filtering surface is less under cyclopropane than it is under ether. Indeed, this must be the explanation until the unlikely possibility that ether increases the permeability of the capillary wall can be ruled out by direct observations. However, the same protein concentration found in the lymph under the two agents supports the view that there is no great change, if any, in capillary permeability under the two agents.

Interesting support, from an entirely different approach, for the view that the

capillary circulation is less extensive under cyclopropane than it is under ether is found in the observations of Zweifach *et al.* (3). They studied the blood flow in the capillary bed of the dog's omentum during graded hemorrhage under several anesthetic agents. Their observations indicated that as bleeding continues under cyclopropane, the number of open capillaries remains normal for one-half hour (average) with constriction (flow through the most direct channels) persisting for the subsequent three hours; whereas with ether, under the same circumstances, the number of open capillaries remains normal for one and one-half hours (three times as long as with the cyclopropane). In the case of ether, the normal period is followed by a relatively brief (45-minute) constrictive phase (one-quarter as long as with cyclopropane) and then an unrestricted condition follows, with widespread flow through all capillaries, under ether but not under cyclopropane.

Anesthetic effects under circumstances where continued hemorrhage is a factor may not be comparable to conditions where this is not the case; however it seems probable that this situation is comparable to the continued stress of surgical procedure with some hemorrhage. It has been the general observation (4) of this group that, under cyclopropane anesthesia, the precapillary sphincters tend to be closed with reduction of the capillary circulation with most of the blood in the major vessels. Here a 'thoroughfare channel' or 'extended arterio-venous anastomosis' serves to transport blood from the arteriole to the venule with less utilization of the capillary bed than with ether. The head of pressure in the thoroughfare channel is greater under cyclopropane with flow more rapid than it is under ether.<sup>1</sup>

These findings are in accord with our observation that more lymph is produced under ether than under cyclopropane anesthesia. Presumably the tissues would be nourished less well under cyclopropane than under ether. Moreover, with the precapillary entrance tending to be cut off, under cyclopropane, and direct routes of flow from arteriole to venule being utilized, the venous flow would be unusually effective in drawing tissue fluid into the venous end of capillaries and less would go to the lymphatics. Under this circumstance, too, less oxygen and other food supply would apparently be available for the distant cells' nutrition than if the tissue fluid followed its usual washing flow from capillary to lymphatic.

Our findings, as well as those of Zweifach and his associates, are in accord with a new interpretation of the often mentioned clinical observation that the venous blood of patients under cyclopropane is unusually well oxygenated. The question can be raised: Is this because the blood proceeded so directly from arteriole to venule that oxygen could not be claimed by the tissues? Is it possible that the tissues under cyclopropane are in actuality poorly oxygenated, poorly nourished? Does this explain the collapse sometimes seen at the end of surgery when patients are taken off the oxygen-rich atmosphere of closed anesthesia?

While lymph flow itself may not necessarily be an important factor in the nutrition of most tissues during anesthesia, there is still the problem of what the effect of an agent might be that reduced lymph flow in tissues where continuous motion is present, that is, in the lungs and in the heart.

<sup>1</sup> Could this explain the often-reported greater bleeding during surgery under cyclopropane than under ether?

Using still another approach and another part of the body we (5) have made observations that are consistent with the view that cyclopropane restricts the capillary circulation more than ether. Under ether in man, the average glomerular filtration rate fell 21 per cent (in comparison with the pre-anesthetic level), and 32 per cent with cyclopropane. Average effective renal plasma flow fell 39 per cent with ether and 52 per cent with cyclopropane. In the 15 men studied, these reductions were always greater under cyclopropane than they were under ether.

#### SUMMARY

When the effects of cyclopropane and ether on lymph production are compared under standardized conditions in dogs, cyclopropane is found to result in the production of less lymph than ether, regardless of which agent is administered first. This is interpreted as reflecting a smaller effective capillary circulation under cyclopropane than under ether. Support for this view is found in the direct observations of the dog's mesentery by Zweifach *et al.* and in the observations of Burnett *et al.* on kidney circulation in man.

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# ALKALI THERAPY OF AMMONIUM CHLORIDE ACIDOSIS IN DOGS<sup>1</sup>

HOWARD W. ROBINSON, ESTHER M. GREISHEIMER, MORTON J. OPPENHEIMER AND WALDO E. NELSON

with the technical assistance of RICHARD MATTHEWS

*From the Department of Physiology, Temple University Medical School*

PHILADELPHIA, PENNSYLVANIA

**I**N SPITE of rather extensive use of alkali (sodium bicarbonate or lactate) by parenteral routes for the prompt correction of severe states of acidosis of metabolic origin, there is lack of agreement concerning its clinical value and even its need. It is generally recognized that, if renal dysfunction resulting from severe states of dehydration can be corrected by increasing the water content of the blood, the kidneys are capable in the majority of instances of effecting the necessary readjustments in the acid-base relationship. The chances of establishing and especially of maintaining such an adjustment are of course increased when the underlying disturbance, e.g. diarrhea or diabetes, is also brought under clinical control.

The speed of recovery from severe states of acidosis has been significantly increased by the prompt administration of alkali by parenteral routes. On occasion it has even seemed that recovery might have been related to the promptness of the correction of the acidosis.

A causal relationship, however, is difficult to prove in clinical practice because of the uncertainties involved in evaluating other variable factors and hence of establishing an adequate control series.

The problem is whether there exists at any time during the course of a severe degree of acidosis a stage when otherwise irreversible or irreparable damage to important cellular structures of the body could be averted by the administration of an appropriate amount of alkali. The experimental work which is described here was designed in the hope that it might be helpful in the ultimate solution of this problem.

## METHOD

Mongrel dogs were used, weighing from 5 to 25 kg. The animals had access to food (hospital scraps) and water *ad libitum* at all times. Severe degrees of acidosis were induced by administration of a per cent ammonium chloride solution by stomach tube. The amount of  $\text{NH}_4\text{Cl}$  given was approximately 100 cc/10 kg. of body weight per dose. Administrations were made at 2-hour intervals, between 10 a.m. and 4 p.m. When emesis occurred shortly after administration, the dose was usually repeated. The total number of days of administration varied. It was continued in each dog until a low pH level (7.10 or less) existed at the end of the day (short duration acidosis), or was maintained throughout the night or longer (longer duration acidosis). Dogs in the 'longer duration acidosis' group required 2 to 10 days of administration of ammonium chloride to meet the required criterion of a morning pH below 7.10 on one or more mornings.

The ease with which an individual dog could be brought into the acidotic state depended in

Received for publication July 8, 1948.

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.

part upon whether it continued to take food or not. Those animals which did so were much more resistant to the development of acidosis. All dogs lost some weight during the course of  $\text{NH}_4\text{Cl}$  administration. Partial fasting and dehydration incident to excessive polyuria were variable contributing factors to this weight loss.

When each dog reached the desired acidotic state, it was either treated with parenteral sodium bicarbonate or was kept as an untreated control. In the treated dogs, the dose of bicarbonate was estimated on the  $\text{CO}_2$  content of serum and body weight (1, 2). When the dogs died or were terminated, the brain, spinal cord and certain peripheral nervous tissues were prepared for subsequent serial section by Dr. Jean K. Weston. Other organs were prepared for routine microscopic study. These findings will be reported later when the time-consuming examinations of serial sections are completed.

Jugular blood samples were collected, allowed to clot and serum removed under oil, attention being given to the precautions recommended by Austin *et al.* (3). The carbon dioxide content and  $\text{pH}$  values were routinely determined and in many cases total base and chlorides were also measured. The determinations of carbon dioxide content were made with the manometric blood gas apparatus of Van Slyke and Neill (4). The  $\text{pH}$  values were obtained with the Beckman glass electrode  $\text{pH}$  meter (model G) held at  $38^\circ \text{C}$ . by an air bath. A hypodermic type of cell was used. Chlorides were determined by the open Carius method (5). Total base concentration of serum was determined by the Polis and Reinhold method (6), which is based on an ion exchange reaction by a cation-adsorbing resin.

## RESULTS

Despite vigorous efforts, the serum  $\text{pH}$  of 18 dogs never could be brought to a level of 7.10 with ammonium chloride by stomach tube, even though administration was continued for periods up to three months. However, in 90 dogs the serum  $\text{pH}$  was brought to a level below 7.10. This latter group forms the basis for this report.

In order to emphasize the rôle of the duration of a depressed  $\text{pH}$  the dogs were separated into *a*) those with a  $\text{pH}$  below 7.10 for 8 to 24 hours (short duration acidosis) and *b*) those with a  $\text{pH}$  below 7.10 for more than 24 hours (longer duration acidosis).

### *Dogs with pH 7.10 or Below for 8-24 Hours (Short Duration Acidosis)*

1. *Untreated Group.* Of these 77.3 per cent (17 dogs) recovered and 22.7 per cent (5 dogs) died. For the first few days of ammonium chloride administration there was a tendency for the  $\text{pH}$  to return to normal values during the night after having been lowered toward 7.10 at the end of the day. The extent of the nocturnal recovery became progressively less as the period of treatment increased. The characteristic pattern of recovery is illustrated in figure 1. Dogs which retained their appetites had a better recovery response than those which did not eat well. The depression of  $\text{CO}_2$  content was seldom below 9 mEq/l. Total base of the serum was usually depressed and serum chlorides always were elevated. The  $\text{pH}$  of dogs which recovered was below 7.10 on one to four occasions, those which died were below this level on only one occasion.

2. *Treated Group.* In this group 80 per cent (12 dogs) recovered and 20 per cent (3 dogs) died. A quantity of 5 per cent sodium bicarbonate was given by vein which was expected (1) to raise the  $\text{CO}_2$  content to 27 mEq/l. The acid-base condition was occasionally determined one to two hours after treatment and regularly on the next morning. The  $\text{pH}$  and  $\text{CO}_2$  content achieved normal values over a number of days. The percentage of dogs recovering was essentially the same in treated

and untreated groups. In no instance was alkalosis produced. Figure 2 shows a typical case which was treated and recovered. Dogs which recovered had  $pH$  values of 7.10 or less one to three times; those which died had such values one or two times.

Data on all dogs with a  $pH$  of 7.10 or below for 8 to 24 hours are included in table 1, A and B.

*Dogs with pH 7.10 or Below for More Than 24 Hours (Longer Duration Acidosis)*

1. *Untreated Group.* 26.3 per cent (5 dogs) of this group recovered while 73.7 per cent (14 dogs) died (without therapy). There was a tendency for a nocturnal return of the  $pH$  from low values toward normal, as described for the group of dogs,

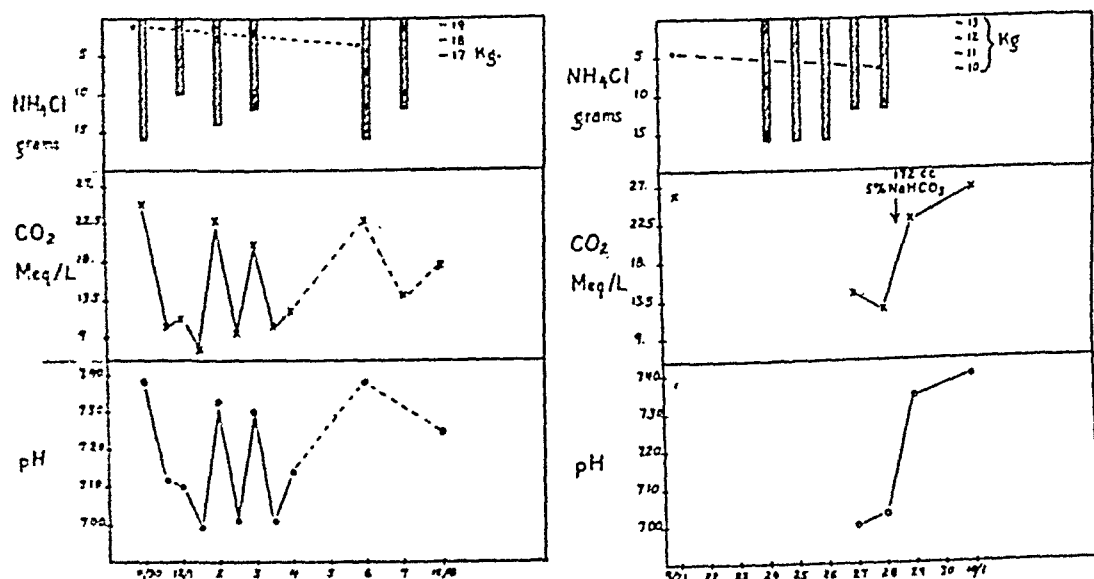


Fig. 1 (left). Dog 9. ACIDOSIS OF LESS THAN 24 HOURS' DURATION. This chart shows three periods of nocturnal recovery from  $pH$  values below 7.10 at the end of the afternoons of Dec. 1-3 to values above 7.10 on the mornings of Dec. 2-4. Dotted lines show recovery without treatment to Dec. 6 when another course of ammonium chloride was started.

Fig. 2 (right). Dog 751. ACIDOSIS OF LESS THAN 24 HOURS' DURATION. First two  $pH$  values are P.M. samples. This chart shows recovery following treatment after second sample on Sept. 28. The last two  $pH$  samples on Sept. 29 and Oct. 1 are A.M. samples.

having a shorter duration of acidosis. Despite the more prolonged period of acidosis depression of  $CO_2$  content was no greater than values in the range of 9 mEq/l. Total base of the serum was more often increased than not and serum chloride was most often increased. Dehydration was progressive as shown by increasing hematocrit values. Dogs of this group rarely ate much, hence weight losses were sometimes severe. It should be noted that about three times as many untreated dogs died if the acidosis persisted more than 24 hours. Figure 3 depicts the data from a typical case which died. The  $pH$  values of all dogs in this group were below 7.10 two to six times.

2. *Treated Group.* Of this group, 55 per cent (14 dogs) recovered and 45 per cent (10 dogs) died. The intravenous bicarbonate therapy was identical with that used in the shorter duration group and  $pH$  values and  $CO_2$  content returned more

TABLE 1A AND 1B. SHORTER DURATION ACIDOSIS

PROCEDURE	CONTROL pH	LOWEST pH	CONTROL CO <sub>2</sub> mEq/l.	CO <sub>2</sub> AT LOWEST pH	CONTROL Cl mEq/l.	Cl AT LOWEST pH	CONTROL TOTAL BASE mEq/l.	TOTAL BASE AT LOWEST pH
Untreated...	7.378 ± 0.045 <sup>1</sup>	7.014 ± 0.062	25.61 ± 1.96	10.40 ± 2.07	105.6 ± 3.7	122.4 ± 6.4	159.1 ± 5.9	154.7 ± 6.2
Recovered...	7.297-7.48 (17)	6.85-7.09 (17)	21.71-28.42 (17)	7.21-14.41 (15)	98.5-110.4 (15)	115.7-136.0 (13)	147-171 (14)	145-164 (10)
Untreated...	7.362 ± 0.048	6.978 ± 0.202	25.00 ± 1.39	12.08 ± 2.64	104.2 ± 3.71	118.1	165 ± 4.4	154
Died.....	7.287-7.40 (5)	6.62-7.10 (5)	23.78-27.39 (5)	9.46-15.73 (4)	100.5-109.5 (5)	111.3-124.8 (2)	160-170 (5)	154 (1)
Treated.....	7.398 ± 0.038	7.037 ± 0.042	24.64 ± 2.28	10.66 ± 2.64	103.5 ± 2.44	122.7	160.6 ± 6.35	159
Recovered...	7.307-7.45 (12)	6.99-7.10 (12)	22.16-28.56 (12)	7.03-13.59 (5)	98.5-106.7 (12)	113.2-132.2 (2)	146-169 (11)	154-164 (2)
Treated.....	7.326	6.863	24.53	6.98	105.4		160	
Died.....	7.27-7.36 (3)	6.83-6.91 (3)	24.28-24.77 (2)	(1)	104.6-106.2 (2)		158-162 (2)	

TABLE 2A AND 2B. LONGER DURATION ACIDOSIS

Untreated...	7.340 ± 0.061	6.942 ± 0.118	23.39 ± 3.88	9.26 ± 2.18	105.3 ± 5.55	121.3 ± 4.78	163	158.5
Recovered...	7.27-7.43 (5)	6.74-7.04 (5)	18.65-28.02 (5)	7.34-12.07 (4)	97.5-110.7 (4)	117.2-127.0 (4)	158-166 (3)	149-168 (2)
Untreated...	7.414 ± 0.046	6.904 ± 0.125	24.01 ± 2.86	6.18 ± 2.52	104.6 ± 5.63	125.3 ± 13.9	160.5 ± 5.72	156.4 ± 15.0
Died.....	7.307-7.47 (14)	6.59-7.08 (14)	20.09-30.22 (11)	2.77-9.28 (6)	94.5-112.9 (11)	103.6-141.8 (7)	147-167 (11)	136-169 (5)
Treated.....	7.364 ± 0.074	6.90 ± 0.111	25.79 ± 2.02	9.86 ± 4.22	105.8 ± 3.5	121.0 ± 11.3	160.8 ± 5.1	155.0 ± 11.8
Recovered...	7.25-7.51 (14)	6.70-7.05 (14)	21.44-27.97 (13)	5.99-19.82 (9)	101.2-111.7 (13)	101.7-133.1 (6)	159-169 (13)	140-170 (5)
Treated.....	7.385 ± 0.074	6.845 ± 0.106	24.76 ± 2.51	8.54 ± 0.43	103.2 ± 3.03	102.3	163.9 ± 4.4	144
Died.....	7.297-7.48 (10)	6.61-7.00 (10)	21.66-29.28 (8)	7.93-8.87 (4)	98.1-111.3 (8)	82.5-127.5 (3)	158-172 (8)	142-146 (2)

<sup>1</sup> Mean with standard deviation; range; number of dogs in parentheses.

rapidly to normal than in control untreated cases which recovered. Chances for recovery are seen to be about twice as good with parenteral administration of sodium bicarbonate as without it when an acidosis at a  $pH$  of 7.10 or below is maintained for more than 24 hours. The data of a typical case which recovered are shown in figure 4. The  $pH$  level of all dogs in this group was below 7.10 two to seven times. Data on all dogs with a  $pH$  of 7.10 or below for more than 24 hours are included in table 2, A and B.

Because it was quite possible that solutions other than 5 per cent sodium bicarbonate might be as effective in promoting recovery of animals with long standing acidosis, a limited series of dogs (ten) was treated with physiologic saline solution and 5 per cent glucose by vein. Since the number of cases is small the exact percentage values are not significant but the data do appear to show that dogs with both short

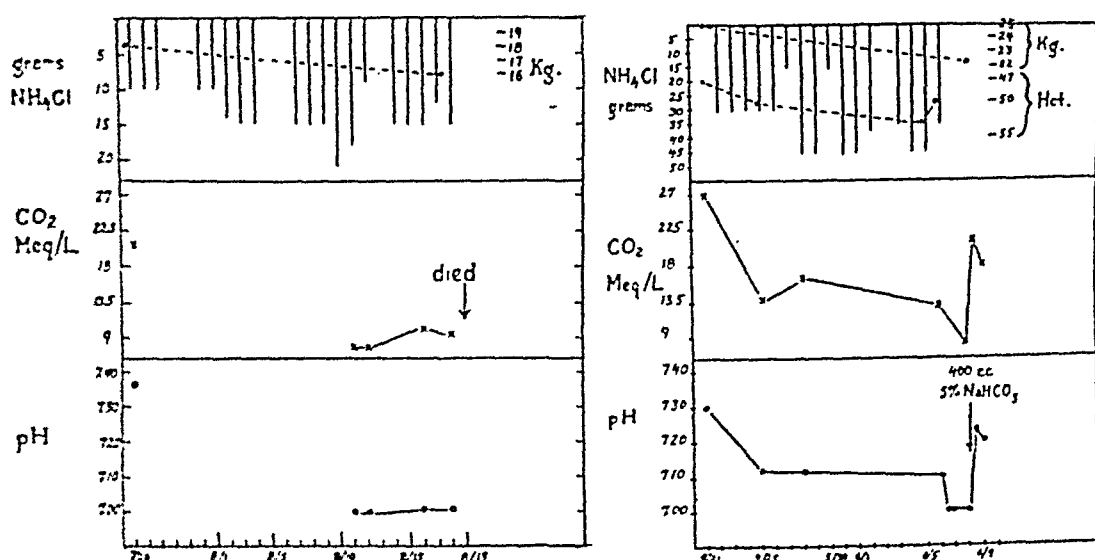


Fig. 3 (left). Dog A. ACIDOSIS OF MORE THAN 24 HOURS' DURATION. This animal died without treatment. First two  $pH$  values are A.M. samples, last two P.M. samples.

Fig. 4 (right). Dog 470. ACIDOSIS OF MORE THAN 24 HOURS' DURATION. This animal recovered with treatment on June 8. Last two  $pH$  values before therapy are A.M. samples.

and longer durations of acidosis recover equally well with glucose and saline solution, as far as ultimate survival is concerned, as with the intravenous administration of a bicarbonate solution. Clinical improvement of these dogs was slower, however, than it was in those treated with bicarbonate. The serum  $pH$  level of the bicarbonate treated dogs was elevated on the first morning following treatment from an average 6.95 to an average of 7.30 in contrast to an average elevation in the glucose and saline treated group of 7.01 to 7.23. The data of these dogs are shown in table 3 which also summarizes tables 1 and 2. It will be noted that the serum  $pH$  of the animals of this group was in the main below 7.10 a fewer number of times and that they did not have the same degree of  $pH$  depression as was obtained in the other two groups.

*Characteristics of the Acidotic State and the Period Immediately after Treatment with Sodium Bicarbonate.* During the first few days of administration of ammonium chloride the dogs urinated frequently and profusely and drank large quantities of

water. Stools became loose, watery and were frequently stained with dark blood. Post-mortem specimens of the gastrointestinal tract from animals which succumbed showed hemorrhages of the duodenum and colon. The eyeballs were sunken, tissues were dry and there was little bleeding during the dissection. As the degree of acidosis increased there were depression of respiration, muscular weakness with ataxia, anorexia and vomiting, and finally anesthesia of superficial and deep structures. General clinical condition and the level of the  $pH$  could not always be correlated. Dogs with a  $pH$  below 7.00 did not always show the degree of clinical symptoms expected, especially during the early period of acidosis. Dogs which died had feeble heart sounds and imperceptible pulses in the agonal period. There was no evidence of disturbances in cardiac rate or rhythm up to the time of demise.

No harmful effects were noted during or after injection of 5 per cent sodium bicarbonate in any case. Loud borborygmi of the intestine were a constant finding during the injection. Dogs which had been comatose seemed to waken and become alert, and were soon walking about after the intravenous therapy with sodium bicarbonate was terminated. The rate of disappearance of symptoms was much less striking with 5 per cent glucose and 0.9 per cent NaCl.

TABLE 3

DURATION OF ACIDOSIS	% RECOVERED UNTREATED	% RECOVERED 5% BICARB.	% RECOVERED GLUCOSE AND SALINE	% DIED UNTREATED	% DIED 5% BICARB.	% DIED GLUCOSE AND SALINE
Less than 24 hours . . .	77.3 (17) <sup>1</sup>	80.0 (12)	75.0 (3)	22.7 (5)	20.0 (3)	25.0 (1)
More than 24 hours . .	26.3 (5)	55.0 (14)	66.6 (4)	73.7 (14)	45.0 (10)	33.4 (2)

<sup>1</sup> No. of cases.

## DISCUSSION

In ammonium chloride acidosis of 8 to 24 hours duration, at  $pH$  7.10 or below, there was little or no difference in the percentage of recoveries under the conditions of these experiments in the groups of dogs which received parenteral 5 per cent sodium bicarbonate solution, 5 per cent glucose and saline solution, or no therapy other than water and food *ad libitum*, respectively. However, when the duration of the acidosis is longer than 24 hours, the administration of 5 per cent sodium bicarbonate solution doubles the chance for recovery. Since in a small series 5 per cent glucose and saline produced just as great a percentage of recoveries as bicarbonate solution, it may be that fluid volume is more important than acid-base reaction. Three times as many untreated dogs died when the acidosis persisted more than 24 hours than when it was less than one day's duration. Apparently irreversible changes occur with much greater frequency after the first 24 hours. These changes might be secondary to loss of intracellular sodium (7) or because of intracellular dehydration, which has been considered to be the cause of death when extracellular fluids are hypertonic (8) as they were in these animals. Similar damage could depend on excessive loss of potassium (intracellular) in the diarrhea uniformly present (9). Dehydration also causes loss of intracellular potassium (10-14) and infusion of ammonium chloride causes extra excretion of potassium from cells (15).

Sunken eyeballs, dry tissues and high hematocrit are all evidences of dehydration. Oral feedings, important to recovery, contain potassium (2, 16, 17) and the dogs which ate their food well resisted both the onset of acidosis and also made a quicker recovery either with or without therapy.

Weakness of skeletal muscle may represent either the effect of an acid  $pH$  depressing the motor cortex (18) or a loss of cellular potassium from muscles incident to severe acidosis and dehydration (19) following the diarrhea (9, 19). In the first instance the paralysis would be central, in the second peripheral. Depression of the sensory cortex by an acid  $pH$  could account for the anesthesia. It was possible to painlessly expose a superficial vein without local anesthesia in the severely acidotic dogs. The lethal  $pH$  level for the intact heart *in situ* is known to be about 6.00 (20). Cardiac arrest occurs suddenly without previous abnormalities in rate, rhythm or conduction at this level of acidosis. Our lowest  $pH$  value was above this critical level and no disturbances of cardiac rate or rhythm were noted. All the dogs showed respiratory depression and not stimulation as occurs in diabetic acidosis. It is possible that the dehydration of the cells of the respiratory center caused the depression since we found extracellular fluids to be hypertonic. This is in agreement with the findings of Winkler *et al.* (8). Furthermore, patients in shock with acidosis do not show hyperpnea (16, 21). This concept is supported by the fact that  $CO_2$  contents were usually above 9 mEq/l. and never below 4.5 mEq/l. as they often are in clinical acidosis. It may be that a respiratory depression with resultant respiratory acidosis is superimposed on the preliminary metabolic acidosis with the result that the  $CO_2$  content is not depressed to the extent which it otherwise would be. The absence of hyperpnea with a low  $pH$  as a result of ammonium chloride acidosis is similar to that seen in man (10) although different from that reported by Haldane (22). Krogh (23) believes that the respiratory center responds to  $CO_2$  as such and not to the hydrogen ion.

The constant presence of borborygmi during the administration of sodium bicarbonate solution may be accounted for in several ways: increased excitability, decreased threshold for nervous stimuli, and increased spontaneous rhythm of the motor cortex (and hence its autonomic representation) when the reaction is made more alkaline (18, 24, 25), direct stimulation of smooth muscle by alkali (26), or exodus of potassium from cells during alkali therapy (7, 19) with resultant stimulation (27) of smooth muscle. Bloody stools probably were the result of the hemorrhagic duodenitis and colitis which Dr. Weston demonstrated at autopsy. The findings in the intestinal tract are similar to those seen in hemorrhagic shock (28).

Acidosis does not involve changes in extracellular fluids alone (19). Our calculations to determine the amount of sodium bicarbonate to be given were based on the assumption that the bicarbonate content of extra and intracellular fluids is equal (1, 2, 17, 29) and made no allowance for depletion of intracellular sodium. This may explain failure of sodium bicarbonate or chloride solutions to fully correct the acidosis in some of the dogs after time for equilibrium was allowed. Moreover, the effect of bicarbonate varies with different patients (1, 17). One type of acidosis resulting from feeding protein milk to premature infants is characterized by almost complete loss of intracellular sodium (17). Intracellular sodium equals about one

tenth of sodium in extracellular fluids, or over one half of extracellular sodium as bicarbonate normally present (17). To fully correct an acid-base disturbance when there is a loss of intracellular as well as extracellular sodium would involve allowance for losses of sodium from the former as well as from the latter compartment. This allowance was not made.

Although 0.9 per cent sodium chloride solution alone is considered inappropriate for the initial treatment of acidosis (17), when used with glucose it increases blood flow and renal function to permit excretion of excess chloride (30, 31). This could explain the effectiveness of the glucose and saline solution in our experiments. In controlled hemorrhagic shock, sodium bicarbonate infusion given during the hemorrhage not only prevents the onset of acidosis but also the development of irreversible shock (28). It has been emphasized that hemorrhagic shock should be considered from the standpoint of an acidosis (32). It has also been shown that sodium bicarbonate protects the kidney against damage from intravenously administered half normal hydrochloric acid (33).

#### SUMMARY

In ammonium chloride acidosis of less than 24 hours' duration as many untreated dogs recover as do dogs treated with 5 per cent sodium bicarbonate or 5 per cent glucose in normal saline. Under conditions of these experiments treatment does not influence the course. In ammonium chloride acidosis of more than 24 hours duration treatment with either 5 per cent sodium bicarbonate or 5 per cent glucose in normal saline doubled the frequency of survival under the conditions of these experiments. Three times as many untreated dogs died when the duration of acidosis was longer than 24 hours. Dogs treated with sodium bicarbonate made a more rapid clinical improvement from severe states of acidosis than did those treated with glucose and saline.

These data indicate that the administration of sodium bicarbonate under the conditions of these experiments is not dangerous but rather is beneficial in relation to speed of recovery.

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# EFFECT OF HIGH PROTEIN AND HIGH CARBOHYDRATE DIETS ON THE ARGINASE AND PHOSPHATASES OF THE LIVER AND KIDNEY OF THE NORMAL AND ADRENAL-ECTOMIZED RAT<sup>1,2</sup>

CHARLES D. KOCHAKIAN, MARY N. BARTLETT AND JEAN MOE

*From the Department of Physiology and Vital Economics, School of Medicine and Dentistry, University of Rochester*

ROCHESTER, NEW YORK

**A**DRENAL cortical extract administered in eight hourly doses will produce a marked increase in the 'alkaline' phosphatase of the fasted adrenalectomized (1, 2) and normal (3) rat. This effect is due to the S-hormones of the extract (3). These steroids, however, will not restore the decreased arginase activity of the liver in the fasted adrenalectomized rat (4) or influence the level in the normal rat (2) in spite of their known (4, 5) and demonstrated (2, 3) glyconeogenic effect. If, on the other hand, the S-hormones or 3 mg/day of desoxycorticosterone acetate are administered for several days, then the reduced liver arginase of the adrenalectomized rat is restored towards normal (6, 7).

It has also been demonstrated that many androgens (8) will not affect either of the above enzymes of the liver in spite of their protein anabolic effect (9) but will increase these enzymes of the kidneys of normal, adrenalectomized (2, 4) castrated and hypophysectomized rats (unpublished data). There are, however, definite species differences in the degree of these responses (10).

Since the 'S' hormones and 'N' hormones are concerned with protein catabolism and anabolism respectively, it seemed that dietary experiments might provide information concerning the significance of the above-mentioned enzyme changes.

## PROCEDURE

Two types of experiments were conducted: *a*) acute, in which the rats were fasted for 24 hours and either allowed to eat *ad libitum* for 9.5 to 10.5 hours or fed by stomach tube at one- or two-hour intervals for eight hours in order to simulate the conditions of the adrenal cortical studies; *b*) chronic, in which the animals were fed the respective diets<sup>3</sup> (table 1) for seven days. A marked excess of thiamine hydrochloride was added to the high carbohydrate diet and of riboflavin, niacinamide and pyridoxine to the high protein diet. It is recognized now that these vitamins are essential for the proper metabolism of these foodstuffs.

The normal rats of the acute experiments and the adrenalectomized-castrated

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Received for publication July 2, 1948.

<sup>1</sup> This investigation was aided by the Josiah Macy Jr. Foundation.

<sup>2</sup> Some of the data of this paper have been reported in the Josiah Macy Jr. Foundation Conferences on Metabolic Aspects of Convalescence, New York, tenth meeting, 72 (1945) and fifteenth meeting, 128 (1947).

<sup>3</sup> The vitamins were generously supplied by Merck and Company.

rats of the chronic experiments were of the Wistar strain from our colony. The normal rats of the chronic experiments were of the Sprague-Dawley-Holtzman strain. The rats were kept in individual metal cages in an air-conditioned room maintained at 78 to 80°F. In the chronic experiments the normal rats were given 10 gm/day of the standard diet for seven days preceding the experimental period. The adrenalectomized-castrated rats had been castrated for four to six months. They were adrenalectomized two months after castration and were maintained at constant body weight by the subcutaneous implantation of a 14 to 15 mg. pellet of desoxycorticosterone acetate<sup>4</sup> and 9.0 to 9.5 gm/day of the standard diet.

At the end of the experiments, the rats were anesthetized with 0.25 to 0.35 ml. of dial-urethane<sup>4</sup>, the livers were removed and weighed on a Roller-Smith torsion

TABLE 1. COMPOSITION OF DIETS

	STANDARD	HIGH CARBOHYDRATE (NO PROTEIN)	HIGH PROTEIN
	gm.	gm.	gm.
Casein. . . . .	16.7		80
Yeast. . . . .	9.2		10
Starch. . . . .		40	
Sucrose. . . . .	61.2	40	
Wesson oil. . . . .	7.4 <sup>1</sup>	2	2
Cellulour. . . . .	1.8	2	2
Cod liver oil. . . . .	2	2	2
Wesson's salts. . . . .	3.7	4	4
Thiamine HCl <sup>2</sup> . . . . .		.004	.001
Riboflavin. . . . .		.002	.008
Niacinamide. . . . .		.010	.020
Pyridoxine. . . . .		.001	.004
Ca pantothenate. . . . .		.010	.010
Inositol. . . . .		.005	.005
Tocopherols <sup>3</sup> (34% concentrate). . . . .	.030	.030	.030

<sup>1</sup> Hydrogenated vegetable oil. <sup>2</sup> Crystalline B vitamins provided by Merck & Co. <sup>3</sup> Provided by Distillation Products Inc.

balance. The left segment of the median lobe was saved for the enzyme determinations (2, 4) and the remainder of the liver was digested in 5 ml. of hot 30 per cent potassium hydroxide (11). The glycogen was precipitated from the digestion mixture by the addition of 20 ml. of 95 per cent ethyl alcohol, the precipitate was hydrolysed in a boiling water bath with 15 ml. of N/1 hydrochloric acid, neutralized to phenolphthalein (12) with 2 N sodium hydroxide and made to volume. Aliquots were analysed for reducing substances by the modified Somogyi method (13) and the results expressed as glucose.

The nitrogen of the urine and of the tissue enzyme homogenates was determined by the micro-Kjeldahl procedure and the urea and ammonia by aeration into 2 per cent boric acid and titration with 0.015 N hydrochloric acid.

<sup>4</sup> The desoxycorticosterone acetate and the dial-urethane were provided by Ciba Pharmaceutical Products Inc.

## RESULTS

*Acute Experiments.* The arginase of the liver (table 2) was not significantly changed by any of the diets in spite of the marked differences in urinary nitrogen and urea excretion. The decreases in units per gram of tissue are due to the increases in

TABLE 2. EFFECT OF DIET ON THE URINARY NITROGEN AND THE ARGINASE ACTIVITY OF THE LIVER OF THE FASTED (24 HRS.) ADULT MALE RAT

DIET	NO. OF RATS	BODY WT.	FOOD INTAKE	URINE NITROGEN		LIVER		
				Total	Urea-N + NH <sub>3</sub> -N	Wt.	Arginase	
							(Total U)	(U/gm.) <sup>2</sup>
		gm.	gm.	gm.	mg.	mg.	%	%
Fasted.....	6	172		51.6		5.762	(60482) (58600-64700)	(10450) (9,900-10,800)
Carbohydrate.....	6	184	10.2	47.6		8.548	-9	-41
Carbohydrate.....	6	192	7.5	30.5	22.2	7.160	-23	-38
Protein.....	6	197	7.9	224.0	181.5	6.983	+8	-15
Standard.....	6	202	10.2	62.7	38.8	8.079	0	-29

<sup>1</sup> After a 24-hr. fast, each rat was given 20 gm. of the prescribed diet from which he ate *ad libitum* for 9.5 to 10.5 hr. before autopsy. <sup>2</sup> Average control values with ranges are given in parentheses.

TABLE 3. EFFECT OF DIET ON THE LIVER GLYCOGEN AND PHOSPHATASES OF FASTED (24 HRS.) ADULT MALE RATS

DIET <sup>1</sup>	LIVER GLYCOGEN	'ALKALINE'		'ACID'	
		(Total U) <sup>2</sup>	(U/gm.) <sup>2</sup>	(Total U) <sup>2</sup>	(U/gm.) <sup>2</sup>
	%	%	%	%	%
Fasted.....	0.432	(10.0) <sup>3</sup> (7.7-13.6)	(1.7) (1.3-2.4)	(146) (135-150)	(25.5) (23.5-28.7)
High carbohydrate (no protein).....	4.212	+40 <sup>3</sup>	-7	+9	-27
High carbohydrate (no protein).....	0.441	-16	-33	-3	-22
High protein.....	1.033	+38	+14	+11	-12
Standard.....	1.177	+43	0	+16	-17

<sup>1</sup> See tables 1 and 2 for details of experimental conditions. <sup>2</sup> Av. control values with ranges are given in parentheses. <sup>3</sup> On activation with MgSO<sub>4</sub> the difference was only +17%.

size of the liver with the deposition of glycogen (table 3). These differences disappear when a comparison of the total enzyme activities is made.

The phosphatases of the liver show indefinite changes. The high carbohydrate diet produced an increase in the first and a decrease in the second experiment. The increase, however, probably is not real for it was reduced to 17 per cent when the levels of the MgSO<sub>4</sub> activated (14) enzymes were compared. The increases obtained with the high protein and the standard diet are only suggestive.

There were no significant changes in kidney weight or enzymes. The greatest change in any of these factors was less than 5 per cent.

The administration of 30 per cent glucose by stomach tube to 24-hour fasted normal rats at one- or two-hour intervals for eight hours increased the liver glycogen, but did not significantly affect the enzymes of the liver or kidney. Data are not shown.

TABLE 4. EFFECT OF DIETS (10 GM/DAY FOR 7 DAYS) ON KIDNEYS AND LIVER OF MALE RAT

DIET	NO. OF RATS	BODY WEIGHT		WT.	NITROGEN		ARGINASE		'ALKALINE' <sup>1</sup> PHOSPHATASE	
		Initial	Change				(Tot. u) <sup>2</sup>	(u/gm.) <sup>2</sup>	(Tot. u) <sup>2</sup>	(u/gm.) <sup>2</sup>
		gm.	gm.	gm.	mg.	%	%	%	%	%
Standard.....	5	249	+1	1.957	29.3	3.05	(226)	(116)	(277)	(142)
High protein.....	5	249	+1	2.200	34.0	3.06	+22	+15	+25	+11
High carbohydrate (no protein).....	5	250	-13	1.611	24.7	3.09	-3	+17	-4	+11

Liver									
	WT.	GLYCOGEN		NITROGEN		ARGINASE		'ALKALINE' <sup>1</sup> PHOSPHATASE	
		Total	%	Total	%	(Tot. u) <sup>2</sup>	(u/gm.) <sup>2</sup>	(Tot. u) <sup>2</sup>	(u/gm.) <sup>2</sup>
	gm.	mg.		mg.		%	%		
Standard.....	8.580	363	4.21	309	3.61	(86900)	(10,200)	(20.7)	(2.4)
High protein.....	9.230	224	2.41	344	3.81	+33	+23	+61	+49
High carbohydrate (no protein).....	7.610	440	5.79	227	2.97	-47	-41	+58	+77

<sup>1</sup> No changes in 'acid' phosphatases of kidney or liver. <sup>2</sup> Av. control values with ranges are given in parentheses.

TABLE 5. EFFECT OF HIGH PROTEIN DIET ON THE WEIGHT AND ENZYMES OF THE KIDNEY AND LIVER OF THE ADRENALECTOMIZED-CASTRATED RAT MAINTAINED WITH DESOXYCORTICOSTERONE ACETATE<sup>1</sup>

DIET	NO.	BODY WT.	KIDNEY			LIVER		
			Wt.	Arginase (u/gm.) <sup>1</sup>	'Alkaline' <sup>2</sup> Phosphatase (u/gm.) <sup>1</sup>	Wt.	Arginase (u/gm.) <sup>1</sup>	'Alkaline' <sup>2</sup> Phosphatase (u/gm.) <sup>1</sup>
			gm.	gm.	%	%	gm.	%
Standard	3	280	1.839	(87) (78-95)	(120) (97-136)	8263	(4,300) (3,880-4,520)	(3.1) (2.8-3.5)
High protein	3	274	2.173	-7	+12	9034	+18	-3

<sup>1</sup> Similar results obtained with incompletely adrenalectomized rats. <sup>2</sup> No effect on 'acid' phosphatases of kidney and liver. <sup>3</sup> Av. control values with ranges given in parentheses.

*Chronic Experiments in Normal Rats.* The normal rats on the high carbohydrate diet lost considerable body and kidney weight but those given the high protein diet maintained their body and increased their kidney weight. The nitrogen (protein) content of the kidneys changed in proportion to their weight changes (table 4). Arginase and 'alkaline' phosphatase levels varied with the kidney weight changes.

The glycogen content and weight of the liver did not parallel each other. The liver of the high protein fed rats was heavier but contained less glycogen than that of

the rats fed the standard diet, while the rats fed the high carbohydrate diet had smaller livers and a greater amount of glycogen.

The nitrogen (protein) content of the liver of the rats on the high protein diet was greater and that of the rats on the high carbohydrate diet was less than that of the rats fed the standard diet.

*Chronic Experiments in Adrenalectomized-Castrated Rats.* The high protein diet increased the weight of both the kidney and the liver of the adrenalectomized-castrated rats, but did not produce any significant changes in the enzymes of these organs (table 5). Similar results were obtained with identically treated but incompletely adrenalectomized rats.

#### DISCUSSION

The results of this study indicate that glyconeogenesis from exogenous protein or carbohydrate either in 'short' 10-hour, or 'long' 7-day, experiments is not accompanied by an increase in the 'alkaline' phosphatase of the liver of rats comparable to that observed during glyconeogenesis from presumably endogenous protein under the stimulus of the S-hormones of the adrenal cortex (1-3.)

The failure of the high protein diet to produce a noteworthy increase in liver arginase is in agreement with the observations of Takehara (15), Kageura *et al.* (16), Lightbody and Kleinman (17), Folley and Greenbaum (7) and Miller<sup>5</sup>. Lightbody and Kleinman (17), however, report that significant increases in this enzyme are obtained if male or female rats are fed a 75 per cent protein diet for three to four weeks. They attribute this to an adaptive phenomenon.

It is particularly noteworthy that the high protein diet did not alter the concentration of the greatly reduced arginase activity of the liver of the adrenalectomized rat. This suggests that the loss in liver arginase activity after adrenalectomy (2, 6, 7) and also probably after hypophysectomy (18, unpublished) is not concerned with urea formation (cf. 3).

The high protein diet produces an increase in kidney and liver weight and protein as expected (19, 20) and a proportionate increase in the enzymes of the adrenalectomized as well as the normal rats. On the other hand, androgens produce a much greater increase in the enzymes, especially arginase, than in kidney weight but no change in the enzymes of the liver.

The decrease in liver arginase after seven days on the high carbohydrate-no protein diet is comparable to that observed by Lightbody and Kleinman (17) in rats fed a 6 per cent protein diet. The 'alkaline' phosphatase, on the other hand, is actually increased and the 'acid' phosphatase is spared in agreement with histochemical studies (21). Thus, the protein starved rat in calling upon its protein reserves (cf. 22, 23) decreases its requirement for arginase but increases it for 'alkaline' phosphatase. The enzymes of the kidneys of these animals were not altered in spite of the decrease in weight and protein content.

There seems to be no general correlation of enzymes with protein content of the liver or kidney. The amount of an enzyme apparently is determined by the specific metabolic demands placed upon the organ (cf. 20, 21).

<sup>5</sup> Personal communication.

## SUMMARY

The feeding of either a high carbohydrate (89%)—no protein, a high protein (casein 80%, yeast 10%) or a 'standard' prepared diet to 24-hour fasted adult male rats for 10 hours caused a deposition of liver glycogen and the expected changes in urinary nitrogen and urea excretion but did not change the activities of the arginase, 'alkaline' ( $pH$  9.8) or 'acid' ( $pH$  5.4) phosphatases of the liver or kidney. The feeding of 30 per cent glucose by stomach tube at one- or two-hour intervals also increased the liver glycogen without any changes in the activities of the liver or kidney enzymes.

The feeding of the above diets for seven days at 10 gm/day to normal 250-gram male rats showed that the high carbohydrate diet caused a loss in body and kidney weight but no change in enzyme activities. The livers of these animals lost weight and protein, but contained a large amount of glycogen. There was a decrease in arginase but a moderate increase in 'alkaline' phosphatase. The high protein diet, on the other hand, maintained the body weight and increased the kidney weight and protein with a concomitant increase in the enzymes. The liver weight, protein and enzymes were somewhat increased. The glycogen content, however, was only one-half that present in the livers of the rats fed the high carbohydrate diet.

The feeding of the high protein diets as above to completely and partially adrenalectomized-castrated rats increased the kidney and liver weights but did not change enzyme activities of these organs. Organ and enzyme changes are not comparable to those after administration of protein anabolic or catabolic steroid hormones.

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# CHOLINESTERASE LEVELS IN PLASMA AND TISSUES<sup>1</sup>

BRUNO MENDEL, ROSEMARY D. HAWKINS AND MARGARET NISHIKAWARA

*From the Banting and Best Department of Medical Research, Banting Institute, University of Toronto*

TORONTO, CANADA

**D**URING the past 20 years frequent attempts have been made to correlate the level of cholinesterase in blood plasma with various pathological conditions, especially with those associated with a disturbed function of the nervous system and of voluntary muscles (1-10). The results of these investigations were often contradictory and on the whole disappointing. Despite the mass of data obtained no conclusions whatsoever could be drawn regarding a relationship between the cholinesterase level of blood plasma and various syndromes. However, at the time when these investigations were carried out, it was not known that there exist, in the animal body, two enzymes capable of hydrolyzing acetylcholine, true cholinesterase and pseudo-cholinesterase (11) and that only the former is essential for the hydrolysis of acetylcholine *in vivo* (12). Moreover, no method was available for distinguishing between these two enzymes and for measuring their activities separately, in sera which, in most species, contain both true cholinesterase and pseudo-cholinesterase in varying proportions. Rabbit serum, for example, contains mainly true cholinesterase, whereas pseudo-cholinesterase predominates in the serum of man (13). Furthermore, in the measurement of the cholinesterase activity of human plasma, it has been customary to use high concentrations of acetylcholine at which the activity of true cholinesterase is depressed while that of the pseudo-cholinesterase is favoured. Therefore, the results of these measurements, though reflecting the level of pseudo-cholinesterase, could give no hint of changes in the level of true cholinesterase, unless changes in the activities of both enzymes follow a parallel course, an assumption for which there has been no experimental evidence.

The present investigation was undertaken to determine 1) whether changes in the activity of the plasma pseudo-cholinesterase entail similar changes in the activity of the plasma true cholinesterase and 2) whether a correlation exists between the level of true cholinesterase in the plasma and that contained in the tissues.

## METHOD AND MATERIALS

The method used for the measurement of cholinesterase (ChE) activity was that of Mendel, Mundell and Rudney (14), who showed that acetyl- $\beta$ -methylcholine is hydrolyzed by true cholinesterase but not by pseudo-cholinesterase and that, conversely, benzoylcholine is hydrolyzed by pseudo-cholinesterase but not by true cholinesterase. With these two substrates it is possible, therefore, to measure the

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Received for publication July 1, 1948.

<sup>1</sup> Aided by a grant from the Banting Research Foundation.



activities of true cholinesterase and pseudo-cholinesterase separately in tissues and body fluids containing a mixture of both enzymes.

Adult, male Wistar rats weighing between 200 to 400 grams were used as experimental animals. They were killed by exsanguination through the jugular vein and the required tissues were removed. The brain was sectioned just below the level of the fourth ventricle so that approximately the same portion of brain was obtained in every case. After removing the blood vessels as completely as possible, a homogeneous suspension was prepared by grinding the tissue with three times its wet weight of distilled water.

Preliminary experiments done on three muscles, sternohyoideus, sternomastoideus and diaphragm, indicated that the former was most suitable as test object. This muscle is easily removed in its entirety and can be readily ground. In addition, of the three muscles, the sternohyoideus showed greatest activity towards acetyl- $\beta$ -methylcholine. No activity was observed towards benzoylcholine, indicating that no pseudo-cholinesterase is present. A 1:3 suspension was used in the experiments to be reported.

One ml. of plasma, one ml. of muscle suspension and 0.25 ml. of brain suspension were tested for their cholinesterase activities by Warburg's manometric method at 37.5°C. in a 0.025 M solution of bicarbonate saturated with 5 per cent  $\text{CO}_2$  in  $\text{N}_2$  (pH 7.4). The final concentrations of acetyl- $\beta$ -methylcholine chloride (Mch)<sup>2</sup> and benzoylcholine chloride (Bch) used in all experiments were 0.6 per cent and 0.15 per cent respectively. The total volume of fluid in the vessel was 5 ml.

#### *Expression of Results:*

a) Activity:  $\mu\text{l CO}_2$  evolved by one ml. plasma in 20 minutes.

b) Q value: 
$$\frac{\mu\text{l CO}_2}{\text{mg. (dry weight)} \times \text{hr.}}$$

### EXPERIMENTAL

#### *Relationship between the Levels of True Cholinesterase and Pseudo-cholinesterase in Plasma*

Before attempting to determine whether or not changes in the activities of the two cholinesterases in plasma follow a parallel course, conditions had to be created whereby the overall activity of the plasma towards acetylcholine would be enhanced or reduced. In preliminary experiments, it was found that thyroidectomy brought about a significant rise in the cholinesterase activity in the plasma of rats (15), whereas starvation effected a considerable decline in the activity. This latter finding is in accord with the observations of McCance, Widdowson and Hutchinson (16), who studied the effects of starvation in man.

a) *Thyroidectomy.* Sixteen male rats were thyroidectomized as outlined by Griffith and Farris (17). They were sacrificed 14 days after operation. The true cholinesterase and pseudo-cholinesterase activities of their plasma appear in section 2 of the table. From a comparison of these results with those outlined in section 1,

<sup>2</sup> Merck's mecholyl.

it can be seen that an elevation of over 200 per cent in the plasma pseudo-cholinesterase activity was effected. The plasma true cholinesterase level, however, did not deviate from the normal. Thus an elevation in the pseudo-cholinesterase level of plasma is not necessarily associated with a corresponding rise in the level of plasma true cholinesterase.

b) *Inanition.* Ten male rats varying in weight from 300 to 400 grams were fasted for a period of 12 days. Water was supplied *ad libitum*. The weight loss ranged from 15 per cent to 28 per cent, with an average loss of 19 per cent. Both true cholinesterase and pseudo-cholinesterase activities of the plasma were tested. A comparison of the results outlined in section 3 with those appearing in section 1 of the table reveals a significant depression in the level of plasma pseudo-cholinesterase, but no change in the level of plasma true cholinesterase. Thus it is evident

TABLE 1. CHOLINESTERASE ACTIVITIES OF PLASMA AND TISSUES

SECTION	ANIMALS	PLASMA				BRAIN		STERNOHYOIDEUS MUSCLE	
		True ChE		Pseudo-ChE		No. of animals	Q <sub>Mch</sub>	No. of animals	Q <sub>Beh</sub>
		No. of animals	Activity toward Mch	No. of animals	Activity toward Bch				
1	Normal	28	63.0 ± 2.1 <sup>1</sup>	27	41.0 ± 2.3	23	22.7 ± 0.7	28	5.64 ± 0.25
2	Thyroidectomized	14	61.3 ± 2.3 t = 0.31 <sup>2</sup> P = 0.76 <sup>2</sup>	16	97.1 ± 8.7 t = 7.7 P = 0.38 × 10 <sup>-12</sup>				
3	Starved	10	64.7 ± 3.3 t = 0.26 P = 0.8	10	21.3 ± 2.7 t = 4.8 P = 2.1 × 10 <sup>-4</sup>				
4	Tumor-bearing	22	25.9 ± 3.2 t = 7.35 P = 0.96 × 10 <sup>-12</sup>			10	23.8 ± 0.5 t = 0.98 P = 0.33	12	5.46 ± 0.29 t = 0.16 P = 0.87

<sup>1</sup> ± represents the standard deviation of the mean.  
<sup>2</sup> 't' and 'P' values are all in relation to values in normal animals.

that a depression in the pseudo-cholinesterase level of plasma is also not necessarily accompanied by a corresponding depression in the level of true cholinesterase.

*Relationship between the Levels of True Cholinesterase in Plasma and in Tissues*

There has been almost universal agreement that debilitating conditions are associated with a reduced ability of the plasma to hydrolyze acetylcholine. Vahlquist (18), Scoz and Cattaneo (19) and Jones and Stadie (20) report very low values in advanced tuberculosis. The latter workers also report depressed activity in advanced cancer. Milhorat (21) in his study of 109 patients chosen at random found that in cases of debilitation there was a depression which varied in a manner parallel with the clinical state. These results were confirmed by Faber (9) who investigated a group of 400 patients.

Many attempts were made in the present investigation to alter the level of true cholinesterase in the plasma of rats. Only in malignancy, however, was a significant deviation in the level of plasma true cholinesterase observed. Therefore, rats bearing a transplanted sarcoma (S39) which ranged in weight from 7 to 50 per cent of

total body weight were used to determine whether a correlation exists between the true cholinesterase levels in plasma and in tissues. The true cholinesterase activities of the plasma, brain and sternohyoideus muscle of these animals appear in section 4. A comparison of these figures with those of section 1 of the table reveals that even though the level of true cholinesterase in the plasma is reduced about 60 per cent, the activity of this enzyme in the tissues remains within the normal range. It would seem, therefore, that a depression in the level of true cholinesterase in the plasma is not necessarily indicative of a decrease in the level of this enzyme in the tissues.

#### SUMMARY AND CONCLUSIONS

Changes in the level of pseudo-cholinesterase in the plasma of male rats do not entail similar changes in the level of true cholinesterase in the plasma. Changes in the level of true cholinesterase in the plasma do not necessarily reflect changes in the level of this enzyme in brain and muscle. Consequently, the activities of the cholinesterases of plasma cannot serve as an index of the level of true cholinesterase in tissues under pathological conditions.

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# NUTRITIVE VALUE OF FRUCTOSE FOR RATS AND EFFECTS PRODUCED ON ITS UTILIZATION BY THIAMINE<sup>1</sup>

CURT P. RICHTER

*From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital*

BALTIMORE, MARYLAND

IN PREVIOUS experiments the single food choice technique was used to determine the nutritive value of glucose and sucrose and the effects produced on their utilization by thiamine (1, 2). Rats of a standard weight were placed on a diet consisting of only one sugar, either glucose or sucrose. The length of time that the rats survived was taken as a measure of the nutritive value of the sugar. Likewise when the rats had access also to a 0.02 per cent solution of thiamine hydrochloride, the increase in the survival time was taken as a measure of the effects produced by this vitamin on the utilization of the sugar. On either glucose or sucrose the rats survived an average of 37 days. When a thiamine solution was available the rats drank it freely and their average survival time on glucose increased to 74 days and on sucrose to 56 days.

These experiments indicated that thiamine has much less effect on the utilization of sucrose than it does on glucose. It was suggested that the presence of the fructose moiety might explain the reduced effect of thiamine on the utilization of sucrose. The following single food choice experiments on fructose were undertaken to test this suggestion.

## METHODS

Female rats, albino or hooded, weighing between 80 and 90 grams were placed in separate activity cages, each equipped with a living compartment and a revolving drum (3). The living compartment contained a non-spillable food-cup and one 100 cc. graduated inverted water bottle. For the next 15 to 20 days, that is until the rats weighed between 120 to 149 grams, they ate a stock diet<sup>2</sup> and drank tap water. Then in one series of experiments the stock diet was replaced with granulated fructose (C.P. Special, Pfanstiehl Chemical Company) and in a second series the stock diet was replaced with a 40 per cent solution of fructose. In the thiamine experiments, the rats had access at the same time to a 0.02 per cent solution of thiamine hydrochloride.

Records were made daily of the food and fluid intake, running activity, as measured by the number of revolutions of the drum, and of vaginal smears. The rats were weighed at weekly intervals. Inspections were made at frequent intervals

Received for publication June 30, 1948.

<sup>1</sup> Work carried out under a grant from the Sugar Research Foundation, New York City.

<sup>2</sup> This diet contained graham flour 72.5%, casein 10.0%, butter 5%, skim milk powder 10%, calcium carbonate 1.5%, and sodium chloride 1.0%.

for signs of nutritive deficiency; all noticeable changes were recorded and photographed.

## RESULTS

**Survival Times.** Figure 1 summarizes the results. It gives the mean survival times (solid lines) and the standard error of the mean (dotted lines) for the rats that received fructose (granulated) without and with access to the thiamine solution. For comparison, it gives the mean survival times for the rats that in previous experiments received glucose or sucrose without and with access to thiamine. The graph shows also the percentage increase in survival times of the rats that had access to thiamine over those that received only the sugars.

**Single Foods—Fructose.** On granulated fructose alone the rats lived longer than they did on either glucose or sucrose. The survival times of the 15 rats on the granulated fructose averaged 45.3 days, which is 8 and 9 days longer than the average for

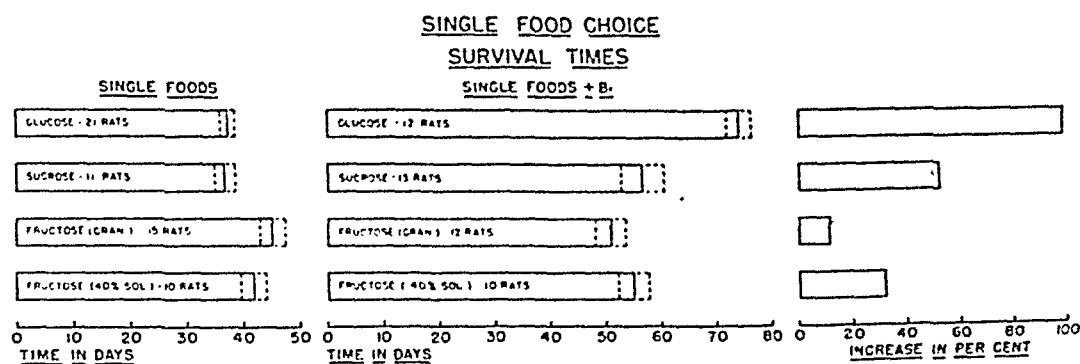


Fig. 1. CHART SHOWING THE AVERAGE SURVIVAL TIMES in days of rats on the single foods without and with access to a 0.02 per cent solution of thiamine hydrochloride; also the increase in per cent produced on the survival times by the thiamine hydrochloride.

glucose (powdered) and sucrose (granulated) respectively. In contrast to the rats on glucose or sucrose that at no time showed any signs of nutritional deficiency, except for emaciation, the rats on fructose showed a marked loss of hair, particularly on the head and over the haunches. The hair, when pulled, came out very easily and in large tufts. The general effect was not unlike that produced by a dietary deficiency of biotin.

It appeared at first that this marked loss of hair resulted from some metabolic effect of the fructose and that, in spite of the longer survival times, as compared to those of the rats on glucose or sucrose, it represented a definite nutritional deficiency. The results of further observations indicated, however, that the loss of hair may depend on an external rather than an internal action of the fructose. It was found that the hair of these animals was sticky to the touch; likewise the wire mesh of the living compartment and revolving drums was sticky. Apparently in some way during eating, the sugar stuck to the hair around the snout or to the paws and from there was distributed to the hair and to the cage, or indirectly from the wire to the hair. In an attempt to eliminate this widespread distribution of sugar to the hair, fructose

was offered to the rats in a 40 per cent solution rather than in granulated form. This meant that the rats could ingest the sugar without getting it on their paws.

Figure 1 shows that the 10 rats on the fructose solution survived on the average of 41.8 days, approximately as long as did the rats on the granulated fructose; definitely longer than did the rats on glucose or sucrose. In marked contrast to the rats on the granulated fructose they showed either no loss of hair at all, or only very slight loss, and in most instances their hair showed no signs of being sticky. Their cages showed only a very slight stickiness, or none at all.

*Granulated Fructose and B<sub>1</sub>.* Figure 1 shows that the 12 rats on fructose with access to the 0.02 per cent solution of thiamine hydrochloride survived on the average 50.7 days, only 6 days longer than on the granulated fructose alone, representing only a 10.6 per cent increase. Like the rats on granulated fructose alone, these

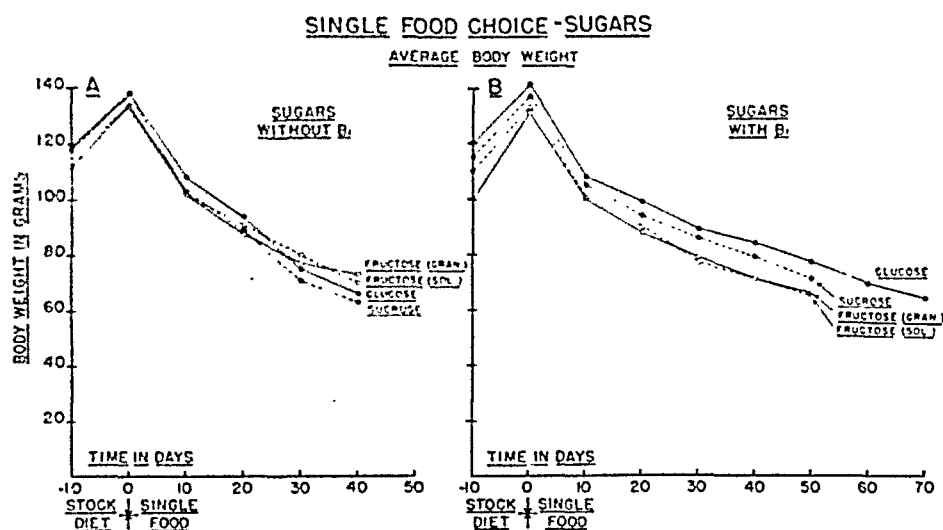


Fig. 2. GRAPHS SHOWING THE AVERAGE BODY WEIGHTS of the rats on the single food choice diets.

rats showed a marked loss of hair over the head and haunches and the presence of a sugary film on the remaining hair.

*Fructose Solution and B<sub>1</sub>.* The 10 rats on the 40 per cent solution of fructose and with access to the B<sub>1</sub> solution survived on the average 55.1 days, not significantly longer than did the rats on the granulated fructose and B<sub>1</sub>. The hair of these rats was not sticky; nor was there any hair loss. The cages showed only a slight tendency to stickiness.

A comparison of the percentage increase in survival times of the rats with access to B<sub>1</sub>, over those that had the sugars only, shows a marked difference between the three sugars. With access to B<sub>1</sub> the rats fed glucose showed a 98.9 per cent increase in survival time; the rats on sucrose a 52.1 per cent increase; the rats on granulated fructose a 10.6 per cent increase; and the rats on fructose solution a 31.8 per cent increase.

*Body Weight—Fructose.* Figure 2 summarizes the results. The curves give the average body weights on the 10th day before the start of the single food diets, the day on which the diet started and, also, the successive 10-day periods. On the single foods without B<sub>1</sub> the curves are much the same, but on the 40th day the rats

on the granulated fructose and those on the fructose solution weighed slightly more than did the rats on either glucose or sucrose. They also lived longer.

*Fructose and B<sub>1</sub>.* With access to the thiamine solution the rats lost weight at a slower rate than did the rats without access to this vitamin (fig. 2B). The rats on glucose lost weight at a slower rate than did those on fructose (granulated or in solution). Here again a direct relationship existed between body weight and survival time.

*Food Intake—Fructose.* Figure 3 gives the average daily food intake in cal/kg. of body weight for the rats on the three sugars without and with access to thiamine. Figure 3A shows that on the stock diet the average daily intake of the four groups of rats ranged from 368 to 419 cal/kg. During the first few days on the single food diets, the rats ate about the same amount of glucose as they had previously eaten

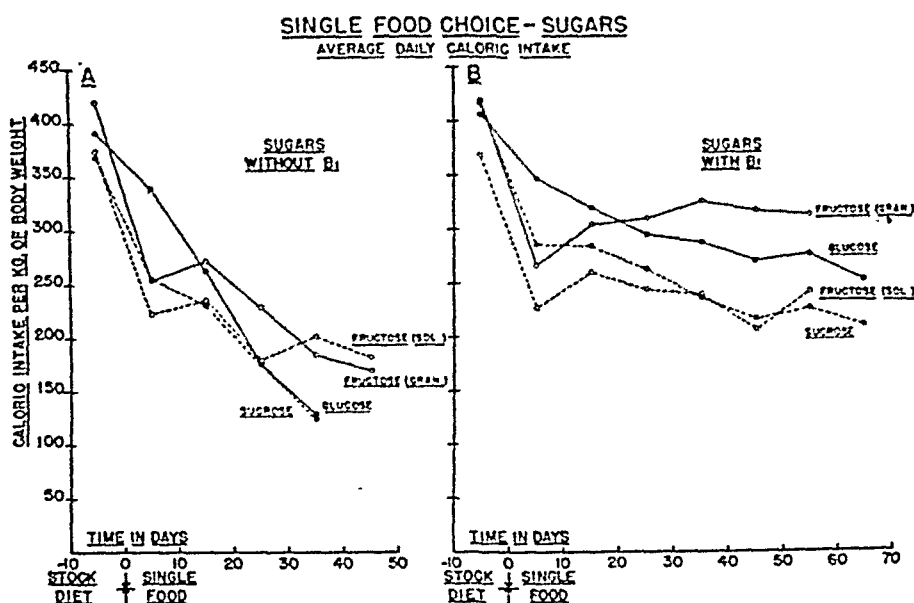


Fig. 3. GRAPHS SHOWING THE AVERAGE DAILY INTAKE in cal/kg. of the rats on the single food choice diet.

of the stock diet; in marked contrast they ate very little or no sucrose or fructose. For the first 10 days the average intake dropped only slightly for the rats on glucose (from 392 to 340), but much more for the rats on sucrose (from 393 to 265) or on fructose (from 419 to 267). Later the rats began to eat more sucrose and fructose and to some extent make up for the initial refusal. The intake of the rats on glucose decreased at a steady rate while that of the rats on fructose showed an increase during the second 10-day period. During the 30- to 40-day period the rats on fructose took much larger amounts than did the rats on either glucose or sucrose. This higher food intake might thus account for the longer survival times and higher average body weights of the rats on fructose as compared to those of the rats on sucrose or glucose.

*Fructose and B<sub>1</sub>.* With the thiamine supplement, just as without, the rats on sucrose and fructose ate very little or no sugar during the first few days after the

change from the stock diet. Figure 3B shows that this brought the average daily intake of these two sugars for the first 10-day period far below that of the rats on glucose. During the next 10-day period the rats on sucrose and fructose either increased their intake or maintained it at the same level. In the subsequent 10-day periods the average of the rats on sucrose, fructose (solution) or glucose decreased at a slow rate, while that of the rats on fructose (granulated) continued to show a very slight increase. In all instances the daily intake levels were far above those of the rats on the sugars without the thiamine supplement. The record for the 50- to 60-day period shows that the intake was lowest for the rats on sucrose

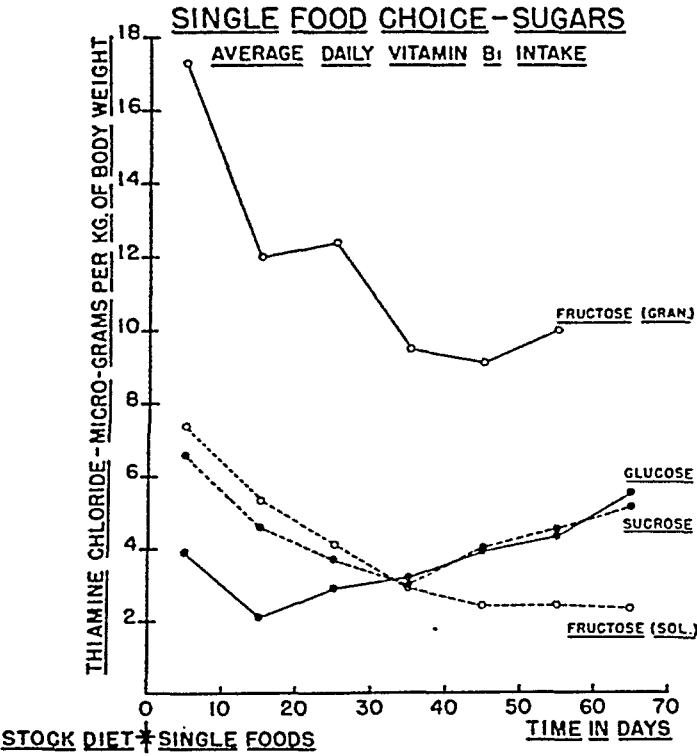


Fig. 4. GRAPH SHOWING THE AVERAGE DAILY INTAKE of thiamine hydrochloride on the regular food choice diet.

and fructose (solution); next came glucose; then fructose (granulated). The actual consumption of granulated fructose was not, however, so high as this figure would indicate, since a considerable amount was diverted to the rats' hair and skin and to the wires of the cages. Leaving out the record of the rats on the granulated fructose the results indicate that the survival times varied with the caloric intake.

*Thiamine Intake.* Figure 4 summarizes the results. It gives the average daily intake of thiamine hydrochloride per kilogram body weight for each group of rats. The curve for fructose in solution parallels that for sucrose during the first 30 days, with both decreasing gradually; thereafter the rats on fructose solution continued to take less and less thiamine while those on sucrose gradually increased their intake, now coinciding with the thiamine intake for the rats on glucose. The glucose rats started out taking much less thiamine than the others and then after an initial drop increased their intakes steadily.



The groups of rats on granulated fructose took very much more thiamine throughout their lives than did any other group. Their curve also shows a gradual though irregular decline. The presence of the large amounts of fructose on the hair and on the cages may in some way account for this higher thiamine intake.

*Vaginal Smears.* No difference in the estrous cycles as determined by the vaginal smears was found between the three sugars. In all instances only one or two four-day cycles were found after the start of the single food diet, without and with B<sub>1</sub>. After that the rats all showed constant diestrous smears.

#### DISCUSSION

The results showed that when granulated fructose is fed as a single food to rats it sticks to their paws and snouts and from there becomes distributed to the hair and skin on the rest of their bodies, also to the wires of their cages. This same tendency was shown by sucrose, but to a much less marked degree; it was not shown at all by glucose.

This finding indicates that fructose must have some property that is definitely less marked or even lacking in glucose. Fructose is about twice as soluble as sucrose (374.78 gm/100 gm. of water as compared to 203.99) and about four times as soluble as glucose (97.51 gm/100 gm. of water) (4), so this property could be a higher solubility. However it absorbs moisture much more readily than does glucose, so this property more likely is a higher hygroscopic action. property involves physical characteristics other than these two.

The presence of fructose on the skin and hair introduces several complications in these experiments. In the first place it may enable bacteria or moulds to grow on the hair and in the follicles and so do damage to the hair; in the second place bacteria or moulds thus nourished may serve as a source of nitrogenous substances and vitamins that the rats may obtain as they lick their hair and skin in cleaning themselves; and in the third place the bacteria thus ingested might change the flora of the digestive tract. It would not explain the failure of thiamine to have as much effect on the utilization of fructose as it does on glucose.

The fact that the rat's hair grows in waves that start from the belly and move up the sides in more or less parallel lines to the back and reach last of all the top of the head and the haunches (5) may explain the localization of hair loss in these places in the present experiment. Any hair lost over these areas would be the last to be replaced.

#### CONCLUSIONS

These single food choice experiments showed that fructose has some property that makes it stick to the paws, snout, hair and skin of rats. The presence of the sugar on the hair in some way promotes the loss of hair especially on the head and haunches. The evidence indicates that the sugar achieves this effect through an external rather than an internal action. Single food choice experiments on fructose are complicated 1) by the fact that not all of the fructose taken from the food receptacles is actually eaten by the rats, some of it being diverted to the skin and hair, and the wires of the cages; and 2) by the possibility that bacteria or moulds may grow

in the fructose and thus supply the rats with vitamins and proteins. Offering the fructose in solution at least in part obviated these complications. Under the conditions of these single food choice experiments the rats lived longer on fructose (solution) than on either glucose or sucrose (41.8 days as compared to 37.2 days and 36.8 days respectively). Access to a 0.02 per cent solution of thiamine hydrochloride increased the survival times of the rats on fructose to 55.1 days, on sucrose to 56.4 days, on glucose to 74.0 days, or 31.8, 52.1 and 98.9 per cent, respectively.

These results indicate that thiamine has less effect on the utilization of sucrose and fructose than it does on the utilization of glucose.

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# EFFECT OF CERTAIN CHOLERETIC AGENTS ON EXCRETION OF PIGMENT AND BROMSULFALEIN IN BILE

A. CANTAROW, C. W. WIRTS, W. J. SNAPE AND L. L. MILLER<sup>1</sup>

*From the Departments of Biochemistry, Physiology and Medicine, Jefferson Medical College*

PHILADELPHIA, PENNSYLVANIA

IN A previous communication (1) we reported observations on the rate of biliary excretion of endogenous and exogenous bile-pigment and bromsulfalein in Thomas-type tubulated duodenal-fistula dogs (2-4), in which normal nutrition and liver function can be maintained for many months to several years. These data serve as controls for the present study of the influence of certain choleretic agents upon biliary excretion of the substances mentioned. There is comparatively little precise information on these points in the literature. Practically none of the pertinent reported studies is entirely satisfactory because of defects inherent either in the analytical methods employed or in the conditions of the experiment. The majority were acute experiments conducted under anesthesia, which affects the flow of bile and, conceivably, the response to a choleretic agent. Others were performed in dogs with the Rous-McMaster type of permanent external bile fistula, which have been found to have, almost invariably, some degree of impairment of liver function, even though apparently healthy (4, 5).

It is felt that the data presented here, to which these objections cannot be raised, represent the response to administration of the choleretic agents employed under as nearly normal conditions as can be attained at the present time.

## *Materials and Methods*

Five trained, cholecystectomized dogs were used, provided with gastric and duodenal fistulae fitted with large cannulae, as described by Thomas (2). The duodenal fistula was placed opposite the ampulla of Vater and bile was collected by inserting a temporary glass cannula (3, 4) into the common duct. The bile was allowed to drain into graduated tubes until the flow became constant. It was then collected in 15-minute samples.

After a one-hour control bile collection period, the choleretic agents employed were injected intravenously as follows: *a*) sodium dehydrocholate, 10 cc. of a 20 per cent solution; *b*) sodium salt of 2-phenylquinoline-4 carboxylic acid (sodium cinchophen), 10 per cent solution, 40 or 50 mg/kg. body weight; *c*) sodium cholate, 10 per cent solution, 50 mg/kg. body weight.

Bilirubin (Eastman Kodak Co.) was injected intravenously in one per cent  $\text{Na}_2\text{CO}_3$  solution (one mg. bilirubin/cc.), in a dosage of one mg/kg. body weight. Bromsulfalein (BSP) was injected in a dosage of 5 mg/kg. body weight. These were injected simultaneously with the choleretic agents.

Bile was collected subsequently in 15-minute samples. Each sample was made up to 5 or 10 cc. with distilled water. Determinations of total pigment in bile were made by the method of Malloy (6) and of BSP by a method described by Cantarow and Wirts (7).

## RESULTS

*Endogenous Pigment Excretion (tables 1 and 2).* The bile volume increased promptly in 2 dogs after intravenous injection of 2 gm. of sodium dehydrocholate,

Received for publication July 27, 1948.

<sup>1</sup> Present address: School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

reaching a maximum in 30 to 60 minutes and returning to the pre-injection level in  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours. The hourly volume increased 271 and 854 per cent in the first hour, and 0 and 297 per cent in the second hour. There was a decrease of 78 and 29 per cent during the third hour. The net three-hour increase was 193 and 1122 per cent, respectively. The bile pigment excretion increased promptly, reaching a maximum in the first 15 minutes, the concentration of pigment falling to a minimum value at 30 minutes. The changes in pigment excretion were as follows: first hour, +90 and

TABLE 1. EFFECT OF CHOLERETIC AGENTS ON BILE VOLUME AND BILE PIGMENT OUTPUT

DOG	CHOLERETIC AGENT		PRE-INJECTION BILE 1 HOUR		POST-INJECTION BILE					
					1st Hour		2nd Hour		3rd Hour	
			Vol.	Pigment	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment
		mg./ kg.	cc.	mg.	cc.	mg.	cc.	mg.	cc.	mg.
3	Sodium dehydrocholate	100	3.5	2.72	33.4	4.15	13.9	2.94	2.5	2.10
5	Sodium dehydrocholate	144	14.0	2.15	52.0	4.10	13.8	2.43	3.1	2.10
1	Sodium cinchophen	50	1.4	2.80	9.4	7.31	6.2	4.83	5.2	7.27
3	Sodium cinchophen	50	2.1	1.87	24.8	8.06	21.6	6.20	20.4	5.35
4	Sodium cinchophen	50	8.2	2.06	21.2	3.78	15.0	4.68	0.8	0.46
5	Sodium cinchophen	40	9.0	2.74	36.0	4.55	18.2	2.61	8.1	1.79
5	Sodium cinchophen	40	3.3	1.00	28.3	8.42	12.7	3.13	5.4	3.02
2	Sodium cholate	50	3.3	2.59	8.5	4.20	0.9	0.67	1.1	4.13
2	Sodium cholate	50	2.8	2.93	13.7	5.46	7.2	6.88	3.4	4.06
5	Sodium cholate	50	7.4	2.17	14.0	3.93	6.2	4.25	6.4	5.92

TABLE 2. EFFECT OF DOUBLE INJECTION OF SODIUM CINCHOPHEN (EACH 50 MG/KG. BODY WEIGHT) ON BILE VOLUME AND BILE PIGMENT OUTPUT

DOG	PRE-INJECTION 1 HOUR		AFTER FIRST INJECTION 1 HOUR		AFTER SECOND INJECTION			
					1st Hour		2nd Hour	
	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment	Vol.	Pigment
	cc.	mg.	cc.	mg.	cc.	mg.	cc.	mg.
3	5.5	3.10	25.6	5.92	24.4	3.94	21.3	3.62
5	5.5	2.28	27.2	5.05	18.1	1.92	17.2	2.31
5	10.5	3.18	43.0	4.70	38.6	3.98	32.8	4.43

+53 per cent; second hour, 0 and +13 per cent; third hour, -23 and 0 per cent; the net three-hour increase was 67 and 66 per cent respectively.

In five instances after injection of sodium cinchophen the bile volume increased to a maximum in 15 to 30 minutes, the choleresis persisting for  $1\frac{1}{4}$ -3+ hours. The hourly changes were as follows: first hour, +158 to +1081 per cent; second hour, +83 to +929 per cent; third hour, -90 to +871 per cent. The net three-hour volume increase was 151-2881 per cent. The bile pigment excretion rose promptly to a maximum within 15 minutes in 4 instances and at 90 minutes in 1 instance. The concentration of pigment fell simultaneously to a minimum level in 30 to 60 minutes. The hourly changes in pigment excretion were as follows: first hour, +66

(in mg/100 gm. of diet): thiamin, 1; riboflavin, 2; pyridoxine, 1; niacinamide, 2; calcium pantothenate, 4; inositol, 200; p-amino benzoic acid, 60; folic acid, 2; biotin, 0.001; and 2 methyl 1-4 naphthoquinone, 0.4. In addition, vitamins A, D, and E were supplied as haliver oil with viosterol fortified with alpha-tocopherol (100 mg/50 cc). Three drops were administered to each rat twice weekly. To this synthetic basal diet various supplements for the different experimental groups, as described below, were added.

TABLE 1. AVERAGE BODY WEIGHTS<sup>1</sup> (IN GM.) OF CONTROL RATS AND OF RATS GIVEN COBALT WITHOUT AND WITH CHOLINE

WKS. ON EXPER.	GROUP			
	Control	Cobalt Alone	Cobalt + 'Low' Choline <sup>2</sup>	Cobalt + 'High' Choline <sup>2</sup>
0	44 ( 38- 50)	45 ( 37- 52)	46 ( 38- 58)	52 ( 45- 62)
1	110 ( 84-143)	77 ( 60- 95)	71 ( 65- 78)	83 ( 71- 96)
2	162 (145-203)	98 ( 80-115)	102 ( 92-118)	86 ( 72- 98)
3	209 (158-253)	113 ( 93-130)	112 ( 94-134)	97 ( 81-113)
4	258 (195-310)	135 (112-165)	132 (113-168)	117 ( 94-138)
5	298 (240-367)	157 (128-203)	155 (131-208)	136 (107-162)
6	325 (275-405)	176 (145-230)	173 (148-238)	166 (134-197)
7	352 (285-453)	195 (160-255)	190 (165-253)	188 (149-218)
8	376 (298-495)	205 (181-258)	201 (178-260)	205 (163-237)
9	398 (318-525)	222 (190-280)	218 (187-283)	222 (174-253)
10	420 (330-553)	237 (198-303)	233 (198-292)	241 (189-279)
11	442 (343-586)	253 (204-325)	249 (220-310)	252 (203-295)
12	463 (353-626)	269 (210-335)	260 (213-343)	266 (212-313)
13	485 (363-660)	285 (214-360)	272 (233-372)	<sup>3</sup>
14	493 (361-675)	289 (208-360)	277 (244-370)	
15	503 (370-685)	301 (225-375)	281 (248-372)	
16	517 (380-698)	309 (228-390)	288 (250-382)	
17	520 (375-704)	314 (225-400)	293 (260-383)	
18	526 (390-698)	326 (240-416)	305 (265-385)	
19	535 (398-702)	333 (240-419)	318 (258-408)	
20	542 (410-706)	347 (260-433)	336 (265-404)	

<sup>1</sup> Minimum and maximum values for individual animals are given in parentheses.

<sup>2</sup> 'Low' level of choline = 2.0 gm kilo diet; 'High' level = 6.0 gm kilo diet.

<sup>3</sup> Group discontinued.

The animals were divided into four groups, the control group, the cobalt group, and two groups receiving cobalt plus choline. Choline was given at two different levels: 2.0 gm. (Davis used 1.91 gm. per kilo diet), and 6.0 gm/kilo diet, respectively. The higher level was used to determine what effect would be obtained with a higher dosage. The control animals received 2 gm. of choline per kilo diet. The other three groups were given 477 mg. of recrystallized  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  per kilo diet. This amount supplies approximately 1.0 mg. cobalt per day. Each group consisted of 12 animals and the experiment was continued for a period of 20 weeks. The body weights of the animals were followed weekly. The food intake was recorded daily and the hemoglobin levels were measured bi-weekly. The hemoglobin values were determined by an acid hematin method employing a Coleman spectrophotometer

calibrated by the O<sub>2</sub> capacity method. Samples of blood were obtained by piercing the dorsal tail vein of the animal.

### RESULTS AND DISCUSSION

From the data in table 1, showing the average body weights for the various groups, it is evident that the control animals grew at a rapid rate reaching a value

TABLE 2. AVERAGE HEMOGLOBIN VALUES<sup>1</sup> (GM. PER CENT) OF CONTROL RATS AND OF RATS GIVEN COBALT WITHOUT AND WITH CHOLINE

WKS. ON EXPER.	GROUP			
	Control	Cobalt Alone	Cobalt + 'Low' Choline <sup>2</sup>	Cobalt + 'High' Choline <sup>2</sup>
Initial	10.5 ( 8.5-12.7)	11.5 ( 8.9-13.7)	11.5 ( 7.0-13.7)	11.3 (10.0-13.3)
2	12.8 (11.3-15.0)	12.5 (12.6-16.4)	14.8 (12.8-17.4)	15.2 (14.0-16.2)
4	13.3 (11.6-14.4)	15.5 (13.1-19.3)	16.6 (13.7-21.2)	16.1 (13.9-17.7)
6	14.7 (13.3-15.9)	16.0 (14.0-18.5)	17.6 (15.1-19.8)	16.8 (13.5-19.1)
8	14.8 (12.8-16.1)	17.3 (15.5-20.2)	17.6 (15.9-19.9)	18.5 (17.9-19.4)
10	14.8 (13.9-15.8)	18.6 (16.1-20.4)	18.9 (16.6-20.4)	18.9 (17.0-19.6)
12	15.2 (13.4-16.3)	19.0 (16.6-20.8)	19.2 (17.2-20.4)	19.5 (17.6-20.4)
14	15.7 (15.2-16.4)	19.6 (17.4-23.1)	19.6 (16.1-20.4)	<sup>3</sup>
16	15.6 (15.2-16.2)	19.6 (17.6-22.7)	19.4 (16.8-22.1)	
18	15.6 (14.8-16.1)	19.7 (17.7-22.7)	19.5 (17.3-20.9)	
20	15.5 (14.8-16.3)	20.4 (19.0-22.4)	20.1 (17.4-21.8)	

<sup>1</sup> Minimum and maximum values for individual animals are given in parentheses.

<sup>2</sup> 'Low' level of choline = 2.0 gm kilo diet; 'High' level = 6.0 gm kilo diet.

<sup>3</sup> Group discontinued.

TABLE 3. STATISTICAL ANALYSIS OF HEMOGLOBIN DATA

GROUP	AV. HEMOGLOBIN	STANDARD DEVIATION	PROBABLE ERROR OF THE MEAN	PROBABLE ERROR OF DIFFERENCE BETWEEN MEANS <sup>1</sup>
Control . . . . .	15.5	±0.17	±0.08	
Cobalt—12 wks. . . . .	19.0	±1.24	±0.24	
Cobalt—20 wks. . . . .	20.4	±0.95	±0.23	
Cobalt + 'Low' Choline—20 wks. . . . .	20.1	±1.33	±0.37	±0.43
Cobalt + 'High' Choline—12 wks. . . . .	19.2	±1.11	±0.25	±0.35

<sup>1</sup> Comparison made with group given cobalt alone for corresponding period of time.

of 542 gm. in 20 weeks. The animals which received cobalt showed obvious indications of interference with body growth. Their weight increased steadily but at a far less rapid rate, attaining an average value of only 347 grams in the same experimental period. The animals of the two groups which received choline with cobalt showed no better growth than those which had received cobalt alone. Their weights followed a course almost directly parallel with those of the cobalt group, reaching weights of 336 for the low-choline group after 20 weeks and 266 grams at the end of 12 weeks for the high-choline group. The latter group was discontinued at the end of 12 weeks since their growth and hematological responses had been the same as those of the group given the lower level of choline. The average daily food intake for the animals

of the various cobalt-fed groups was uniformly between 12 to 14 grams per day; the controls consumed a slightly higher level, 15 to 16 grams.

The average hemoglobin data given in table 2 show the expected steady increase with age in all of the groups. From an average range of initial values of 10.5 to 11.5 grams per cent, the levels of each group increased until approximately the 12th to 14th week of the experimental period, then stabilized. Thus the control rats attained a constant average value of about 15.5 grams per cent, while the cobalt-fed group reached a level of approximately 20 grams per cent. Both groups receiving choline with cobalt also showed an average hemoglobin value of approximately 20 grams per cent. In table 3 are given the results of a statistical analysis of the hemoglobin data. It is evident that there is no significant difference between the values for the animals given cobalt alone and those supplemented with choline at either level.

The present data thus show no demonstrable effect on the polycythemia produced by cobalt from supplements of choline given at either of the dosage levels employed. These data are therefore not in agreement with those obtained by Davis in the dog. It is rather difficult to explain the apparent discrepancy between the results unless it be one of a species difference in response to choline administration. However, Best's report (4) that choline administered orally to the dog does not produce an anemia as has been claimed by other investigators (5, 6) likewise indicates the absence of a demonstrable effect of choline on hemopoiesis in this species at the dosage levels employed. The recent observations of Kunkel and co-workers (7), that the parenteral administration of acetylcholine to dogs does not produce an anemia as has been claimed by others (6), is also interesting in this connection.

#### CONCLUSIONS

Polycythemia, as evidenced by a marked increase in the hemoglobin level, was produced in rats by the continued oral administration of a small amount of cobalt as a supplement to an adequate, synthetic diet. The cobalt-fed animals grew at a decreased rate and the average hemoglobin level reached approximately 20 gm/100 ml. of blood.

The administration of choline, at a level of either 2.0 or 6.0 gm/kilo diet, with cobalt resulted in the same retardation of growth and the development of a polycythemia to the same extent as found in rats given cobalt alone.

These results are therefore not in accord with the claim that, in the dog, choline prevents the production of polycythemia by cobalt.

Appreciation is expressed to Dr. Thomas H. Jukes, Lederle Laboratories, for a generous supply of folic acid.

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# SOME PHYSIOLOGICAL EFFECTS ASSOCIATED WITH CHRONIC CALORIC RESTRICTION<sup>1</sup>

ROSWELL K. BOUTWELL, MIRIAM K. BRUSH AND HAROLD P. RUSCH

*From the McArdle Memorial Laboratory, University of Wisconsin Medical School*

MADISON, WISCONSIN

THE inhibiting effect of caloric restriction on the formation of experimental tumors is well established (1, 2), but little is known concerning the mechanism by which this occurs. As early as 1914 Rous (3) observed that the development of mammary tumor transplants and metastases in the mouse was delayed by food restriction and he suggested that this effect was due to a delay in the vascularization and in the development of a supporting stroma upon which the tumor is dependent. The inhibition of the formation of spontaneous mammary cancer in mice on a reduced food intake has been explained on the basis of a pituitary insufficiency producing a decreased ovarian secretion (4). Thus, one of the factors essential for the occurrence of this type of tumor is deficient when caloric intake is restricted. However, the mechanism of the inhibiting effect of simple caloric restriction on the development of other types of neoplasms remains obscure.

In order to obtain further information on this problem, a preliminary survey of certain organ weights (pituitary, thyroid, adrenal, thymus, ovary, uterus, heart, liver and kidney) was made on groups of 12 to 16 mice kept on *ad libitum* or restricted diets for one week, one month and seven months. The results confirmed the conclusion that a reduction in caloric intake decreased ovarian function and in addition suggested that it increased adrenal cortical activity. In order to obtain further information concerning a possible adrenal stimulation, several criteria that indicate the activity of this organ were investigated. These included data on the weight and ascorbic acid content of the adrenals, the content of glycogen in the liver under different conditions and the activity of the lymphatic system as measured by the weight of the thymus and by lymphocyte counts.

## METHODS

Male and female mice of the ABC and Rockland strains, 2 to 3 months of age, were used. Mice of only one strain and one sex were used in any particular experiment. Groups of 6 to 18 mice were kept in screen bottom cages and fed from a special type of feeder (5) either at *ad libitum* (generally 10 to 12 cal/mouse/day) or at restricted levels (6 to 7.2 cal/mouse/day). The diet was weighed into the feeders daily and the mice were fed at about 9:00 A.M. The composition of the artificial type diets is shown in table 1. Water was available to both groups at all times.

Received for publication July 22, 1948.

<sup>1</sup> This investigation was supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council and by a grant from the Wisconsin Division of the American Cancer Society.



Samples for organ weight, liver glycogen, blood sugar and adrenal ascorbic acid determinations were taken from mice as soon as the corneal reflex disappeared following the injection of sodium pentobarbital. Blood was withdrawn from the right ventricle of the exposed heart with a syringe moistened with a solution of the sodium salt of heparin (10 mg/cc). The weights of organs under 50 mg. were quickly determined to 0.02 mg. on a torsion balance. Liver samples were dropped without delay into a tared tube containing 2.0 cc. of 30 per cent alkali and glycogen digests were made according to the method of Good, Kramer and Somogyi (6). Glucose determinations on the digests and on deproteinized blood filtrates were made by the iodometric copper method of Somogyi (7). The liver glycogen values were expressed in terms of the glucose equivalent. The adrenal ascorbic acid determinations were made by the method of Roe and Kuether (8). Tail blood was used for the total white cell counts. The estrus cycle was followed by the usual vaginal smear technique.

TABLE 1. COMPOSITION OF DIETS

	AD LIBITUM	RESTRICTED
Cerelose.....	78	36.6
Casein.....	15	15
Corn oil.....	2	2
Liver conc.....	1	1
Salt mix <sup>1</sup> .....	4	4
	100	58.6

Vitamins added: mg/15 gm. of casein; thiamine hydrochloride, 0.3; riboflavin, 0.3; pyridoxine hydrochloride, 0.3; niacinamide, 0.5; calcium pantothenate, 2.0; inositol, 25; p-aminobenzoic acid, 25; choline chloride, 50; pteroylglutamic acid, 0.1; and biotin, 0.01. Halibut liver oil was added to the corn oil at the level of 7500 vitamin A units and 108 vitamin D units per kg. (6 drops x kg. of diet).

<sup>1</sup> PHILLIPS, P. H. AND E. B. HART. *J. Biol. Chem.* 109: 657, 1935.

#### EXPERIMENTAL AND RESULTS

*Liver Glycogen and Blood Sugar.* The diurnal fluctuation of liver glycogen and also blood sugar was followed in mice adapted to the restricted and *ad libitum* diets. Since the feeding habits of these mice differed, this variation must be considered in relation to the results. Mice, restricted in food, consumed their daily allotment in less than one hour after the 9:00 A.M. feeding time. However, six hours later their stomachs were still distended with ration, which was mostly in an undigested, semi-dry state. Even 12 hours after feeding time considerable amounts of partially digested food were still present, but by 24 hours the stomach was empty. The mice that were allowed the full ration were also fed at 9:00 A.M., but the major portion of their food was eaten between 6:00 P.M. and midnight. At no time was the stomach distended nor was a dry mass of diet found therein.

After an equilibration period of four weeks or longer, the content of glycogen in the liver was determined in mice taken from the two groups at 3:00 P.M., 9:00 P.M. and 9:00 A.M. the following morning (6, 12 and 24 hours after feeding time, respectively). The results are plotted in figure 1. Each point is an average of 19 or 20 mice in the curve representing the restricted group and of 6 mice to a point in the

curve depicting the mice on the *ad libitum* diet. The same data were replotted for the second 24-hour period to emphasize the cyclic nature of the changes. This diurnal variation in liver glycogen of restricted animals was apparent by the fourth day after caloric restriction was begun and reached the extremes similar to those shown in figure 1 by the tenth day, after which there was no change for as long as 193 days. The unusually high level of liver glycogen in the restricted mice 6 hours after feeding

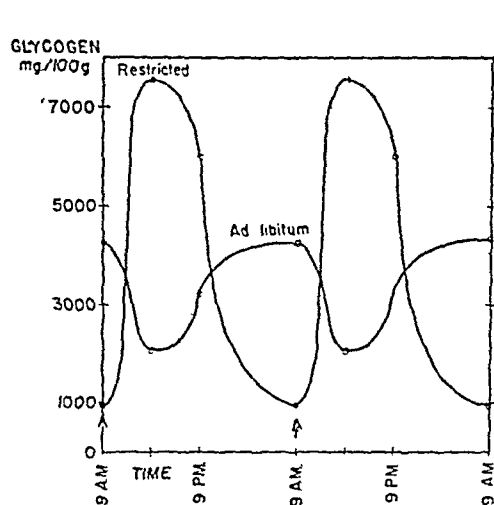


Fig. 1

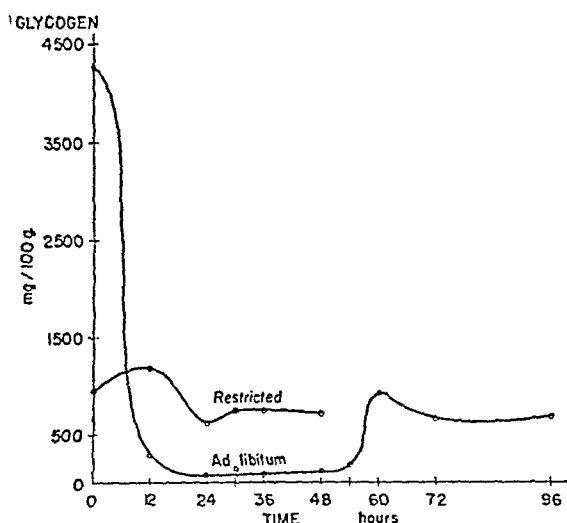


Fig. 2

Fig. 1 (left). DAILY CYCLICAL VARIATION in the amount of glycogen in the livers of mice maintained on *ad libitum* and restricted diets.

Fig. 2 (right). EFFECT OF A PROLONGED FAST on the level of glycogen in the livers of mice maintained on *ad libitum* and restricted diets. The last food was given 24 hours prior to the zero time of these curves, and the zero time of this figure corresponds to the points at 9 A.M. on the curves in figure 1. The fast was continued until the death of the animals.

TABLE 2. AVERAGE LEVEL OF BLOOD SUGAR OF MICE ADAPTED TO RESTRICTED AND *AD LIBITUM* FEEDING HABITS AT 6, 12 AND 24 HOURS AFTER FEEDING TIME. THE NUMBER OF ANIMALS PER GROUP IS SHOWN IN PARENTHESES

TIME	RESTRICTED mg. %	AD LIBITUM mg. %
6	101.4 (7)	114.5 (8)
12	65.1 (6)	91.7 (7)
24	97.4 (8)	158.8 (8)

was of the same magnitude as that reported by Long and his associates (9) for mice given injections of cortin and allowed ration *ad libitum*.

At the same time that the mice were sacrificed for the glycogen determination, blood was obtained for the analysis of sugar. There was a daily variation with the feeding habits of the mice in both groups, yet the well fed mice showed a consistently higher level of blood sugar (table 2). This finding is in agreement with the earlier observation of Rusch, Johnson and Kline (10).

It is well known that the administration of certain adrenal cortical steroids to a fasting mouse will prevent the expected depletion of liver glycogen reserves (9). This principle was the basis for an experiment to test the endogenous production of cortical hormones by mice on restricted and *ad libitum* feeding habits. The fast was

begun by removing the food containers from the cages of both groups of mice at 9:00 A.M., 24 hours after the last feeding time, and accordingly this point is represented as zero time in the presentation of the data (fig. 2). It also follows that the glycogen values at this time are the same as at 9:00 A.M. in the experiment described in the preceding paragraphs. In the case of mice on the *ad libitum* regimen, the level of liver glycogen rapidly fell to values below 100 mg. per cent and it remained at this low level for 60 hours when it increased to about 700 mg. per cent, an observation similar to that made by Mirski and his associates (11). This probably indicated stimulation of adrenal cortical activity after the stress of an acute starvation. In contrast, the content of glycogen in the liver of restricted mice never decreased below the zero time level of 700 mg. per cent and was indicative of a high level of cortical activity prior to the period of prolonged fast. The length of the experiment was determined by the survival time of the mice in each group and the mice with the greater reserves lived the longer.

A more refined test of endogenous cortical activity was devised by an adaptation of the adrenal cortical assay method of Venning, Kazmin and Bell (12). They found

TABLE 3. EFFECT OF CALORIC INTAKE AND OF A GLUCOSE SUPPLEMENT UPON THE DEPOSITION OF LIVER GLYCOGEN (MG. OF GLYCOGEN/100 GM. LIVER)<sup>1</sup>

AD LIBITUM CONTROL	AD LIBITUM PLUS GLUCOSE	RESTRICTED CONTROL	RESTRICTED PLUS GLUCOSE
30	62	623	2250
32	68	888	2385
36	214	892	2420
64	493	1320	2450
—	—	—	—
41 av.	209 av.	931 av.	2376 av.

<sup>1</sup> Four mice in each group.

that the injection of small amounts of glucose such that there was no increase in the amount of liver glycogen in adrenalectomized mice increased several fold the sensitivity of the Reinecke-Kendall (13) method for the assay of cortin preparations. In the present study, the effect of small amounts of glucose on the level of liver glycogen was determined in fasted Rockland mice previously adapted to the two diets. Two intraperitoneal injections of 0.3 cc. of a 5 per cent glucose solution were given 32 and 33 hours after the last feeding time (corresponding to 8 and 9 hours after zero time in fig. 2). A total of 30 mg. of glucose was given to each mouse. The content of glycogen in the liver of both the glucose injected and control mice was determined three hours after the second injection and the results of a typical experiment are shown in table 3. The control mice did not receive glucose and the glycogen values are comparable to the 12-hour values shown in figure 2. It is noted that the administration of the glucose increased the glycogen in the liver to an average of 2376 mg. per cent in the restricted mice, but had a very minor effect on the livers of mice accustomed to a diet of abundant calories. Since the average weight of the livers is 1 gm. in the mice on the restricted diets and 1.5 gm. for those on the *ad libitum* ration, it can be seen that about 48 per cent of the total glucose administered was converted to glycogen in the underfed mice as compared to only 8 per cent in the fully fed animals.

**Adrenal and Thymus Weights.** In a typical experiment, 12 male mice of the ABC strain 2½ months old were restricted to six calories daily for five weeks and 8 mice were allowed ration *ad libitum* (between 10 and 11 cal. per mouse daily). The average weight of the mice at five weeks, the average weight of the glands, and the weight of the glands expressed as mg/gm. of body weight are shown in table 4. There was no difference in the absolute weight of the adrenals, but the adrenal weight relative to the body weight was more than half again as large. These findings agree with those reported by Quimby (14) for underfed young male rats but differ from those of Mulinos and Pomerantz (15). The latter investigators found an increase in adrenal weight in rats on complete starvation but a decrease in the weight of the adrenal when the animals were kept on a diet restricted by 50 per cent. The present

TABLE 4. BODY WEIGHTS AND WEIGHTS OF ADRENAL AND THYMUS GLANDS AFTER 35 DAYS OF RESTRICTED AND AD LIBITUM CALORIC INTAKE

	RESTRICTED	AD LIBITUM
No. of mice.....	12	8
Av. body wt.....	16.7 gm.	26.0 gm.
Av. adrenal wt.....	2.38 mg.	2.36 mg.
Wt. ratio, adrenal/body.....	0.144 (0.022) <sup>1</sup>	0.091 (0.010) <sup>1</sup>
Av. thymus wt.....	3.86 mg.	25.2 mg.
Wt. ratio, thymus/body.....	0.236 (0.155) <sup>1</sup>	0.972 (0.168) <sup>1</sup>

$$^1 \text{ Standard deviation } (S = \sqrt{\frac{\sum x^2}{(N-1)}}).$$

TABLE 5. EFFECT OF CALORIC RESTRICTION ON THE WEIGHT OF THE OVARIES AND THE UTERUS

	RESTRICTED	AD LIBITUM
No. of mice.....	6	8
Av. body wt.....	20.7 gm.	28.6 gm.
Av. ovarian wt.....	8.39 mg.	20.5 mg.
Wt. ratio, ovarian/body.....	0.408	0.717
range.....	(0.281-0.606)	(0.452-1.06)
Av. uterine wt.....	18.5 mg.	86.8 mg.
Wt. ratio, uterine/body.....	0.903	3.07
range.....	(0.361-1.85)	(1.23-5.47)

experiment also showed a striking involution of the thymus gland of restricted mice which amounted to a factor of seven on the actual weight basis and of four on the relative basis (table 4).

**Lymphocyte Counts.** The average total leukocyte and lymphocyte count of 6 ABC control mice fed *ad libitum* were 10,300 and 8000 respectively, and of 16 mice restricted in calories, 5700 and 1900. The decreased lymphocyte count was largely responsible for the lower total counts found in restricted mice.

**Adrenal Ascorbic Acid.** The ascorbic acid content of the adrenals of the strain ABC mice on the two diets was also determined. There was no difference between the two groups. Data from a typical experiment showed that this value averaged 234 mg/100 gm. of adrenal (s.d. 9.4) for 8 restricted mice and 237 mg/100 gm. (s.d. 17.6) for 8 mice on the full fed diet.

**Ovarian and Uterine Weights.** For this experiment 8 young adult female mice

of the Rockland strain were fed *ad libitum* and 6 were restricted in calories to 60 per cent of the other group. After 53 days the ovarian and uterine weights were determined and the ratio of these weights to body weights was calculated. The data, presented in table 5, showed that average weight and the weight ratio of these two organs was reduced by 40 per cent or more in caloric restriction.

As evidenced by the vaginal smear technic, estrus ceased in all mice restricted to six calories daily, but was normal in all mice allowed 10 or more calories of the control ration. In order to determine whether the anestrus may have resulted from a decreased production of estrogens or to an inability of the tissues to respond to the hormone, the mice on the restricted diet were injected subcutaneously with 0.08  $\mu$ g. of estradiol benzoate. Estrus resulted in all the mice which proved that the vaginal epithelium was still capable of responding to this stimulus. This is a confirmation of an earlier short-term experiment by Mulinos *et al.* (16) in which estrus was restored in rats subjected to complete starvation following the injection of either estradiol monobenzoate or gonadotropic hormone (Follutein).

#### DISCUSSION

Direct methods for the measurement of pituitary-adrenal-cortical activity have yet to be developed for so small an animal as the mouse. However, there are several measurable physiological criteria that indicate the state of this system. An involution of the thymus gland (17), a decrease in the lymphocyte count (18), an increase in the weight of the adrenal gland (19, 20) and a decrease in its content of ascorbic acid (21), and an increase in gluconeogenesis (9) are some of the manifestations of an intensified adrenal cortical activity. The finding of these changes in the mice restricted in calories strongly support the conclusion of a stimulated cortex in this condition. The one exception was the lack of a measurable change in the ascorbic acid content of the adrenal. Apparently the decrease in ascorbic acid is manifest only after conditions of acute stress (22), since similar changes have not been reported during the mild chronic stress described as Type II by Sayers *et al.* (21).

The experiments in which liver glycogen was determined in fasting mice previously adapted to the two diets and given small glucose supplements are particularly significant. The fast eliminated the cyclic effect of food consumption on the amount of glycogen (fig. 1) and permitted a greater spread in the glycogen content following the administration of low levels of glucose to the two groups. By standardizing the response in liver glycogen to that obtained with known amounts of cortical steroids, this technique is being developed for the assay of the endogenous production of hormones of the adrenal cortex.

The high content of glycogen in the livers of mice on a low caloric intake probably arises in part from non-carbohydrate sources such as amino acids and protein. These adrenal controlled glycogen precursors can be designated as the building block reserve. In contrast, the well fed mouse has a rate of cortical steroid secretion which apparently causes the conversion of only negligible amounts of this reserve to carbohydrate, as is shown by the lower content of liver glycogen. Perhaps this regulation by the adrenal explains the inhibiting effect of caloric restriction on tumor formation. In the restricted animal all available nutrients may well be required for the maintenance

of life, but in the well fed animal there is sufficient energy to satisfy the critical requirements and still allow a reserve of building blocks that could be readily available to respond to carcinogenic stimuli with the ultimate appearance of a tumor. These conditions apply only during the early stages of tumor development and have little effect after the establishment of an independent blood supply to the neoplasm.

The results of this experiment appear to be the first demonstration of an activated pituitary-adrenocortical mechanism in mice on prolonged caloric restriction. In contrast, the decreased ovarian and uterine weights and the cessation of estrus in the restricted mice reflect a lowered secretion of the gonadotropic hormones, a conclusion also reached by Huseby and his associates (4). This variance in the elaboration of two of the hormones of the pituitary suggest that the 'general adaptation syndrome' described by Selye (23) might be operative in the mouse restricted in calories with a shift to the production of the essential adrenocorticotropin at the expense of the less critical hormones. Accordingly, the term pseudohypophysectomy used by Mulinos and Pomerantz (24) to describe the condition in chronic or complete starvation cannot be used to accurately characterize the condition of the pituitary in the mouse chronically restricted in calories.

Although mice on restricted diets were in anestrus, it is significant that they respond normally to physiological amounts of estradiol. This demonstrated that the end-organ response was not impaired and that cell proliferation was not incompatible with caloric restriction.

#### SUMMARY

The state of activity of the adrenal cortex and of the ovary and uterus was investigated in two groups of young adult albino mice maintained for long periods on diets varying as to caloric value. One group was allowed a diet *ad libitum* while the other group was restricted in calories to 60 per cent of the other.

The mice on the restricted diet were in anestrus, but they responded normally to physiological amounts of estradiol. Thus, cell proliferation was still compatible with caloric restriction. The study of the estrus cycle together with the determination of ovarian and uterine weights indicated a lowered ovarian hormone production in mice restricted in calories. In contrast to this, these same mice showed evidence of an increased activity of the adrenal cortex; the involution of the thymus, the decrease in lymphocytes, the increase in the ratio of the adrenal to body weight and the increase in gluconeogenesis all favored this concept. The apparent variance in the secretion of the gonadotropic and adrenocorticotropic hormones from the pituitary suggested that the 'general adaptation syndrome' might be operative in mice restricted in calories, i.e. a shift by the pituitary to the elaboration of the essential adrenocorticotropin at the expense of other less critical hormones. The relation of these findings to the inhibiting effect of caloric restriction on tumor formation is described.

We are indebted to Professor R. K. Meyer of the Department of Zoology of the University of Wisconsin for assistance with some preliminary work in this problem. We also express our thanks to Dr. Augustus Gibson of Merck and Company for a generous supply of the B vitamins and to Dr. B. L. Hutchings of the Lederle Laboratories for the pteroylglutamic acid used in this study.

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# STUDY OF IN VITRO METHODS FOR THE DEMONSTRATION OF ISO-AGGLUTINATION WITH THE BLOODS OF NORMAL AND OF ILL DOGS

ANGIE S. HAMILTON

*From the Harrison Department of Surgical Research, Schools of Medicine,  
University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

IN THE course of an investigation of the source of the factor responsible for the individual specificity of normal dog plasma or serum (1) and the relationship of this factor to the urticaria elicited by infusions of plasma from other dogs, an additional syndrome resembling anaphylactic shock was observed occasionally (2). While all dogs were sensitive in varying degrees to the urticaria-producing factor, anaphylactic shock was not necessarily produced in each of two recipients. *In vivo* hemolysis was not demonstrable in the reacting dog. Occasionally the reaction was detected only with the aid of certain laboratory criteria, particularly leucopenia and delayed disappearance of injected bromsulphalein. The phenomenon appeared unexplainable in certain instances except on the basis of blood group incompatibility, with the inclusion of soluble agglutinin or natural iso-agglutinins in the injected plasma.

It is generally believed that distinct iso-agglutination does not occur with normal dog blood (3) and that preliminary blood grouping in dogs is unnecessary for ordinary experimental procedures. Isohemolysis and iso-agglutination have been observed, however, following sensitization by repeated transfusions of whole blood or washed erythrocytes (4-6). Using iso-immune sera, von Dungern and Hirsfeld (7) and Brockmann (8) were able to establish the existence of two agglutinogens in dog erythrocytes, giving rise to four blood groups.

The presence of natural iso-agglutinins in dog serum was reported in 1913 by Ottenberg, Kaliski, and Friedman (4) and was later confirmed by McEnery and associates (9). The success of the agglutination test was attributed by the originators to the fact that saline solution was not added to the reacting system which consisted of one volume of defibrinated blood mixed with 19 volumes of serum. The test was made at room temperature since incubation at 37° C. resulted in hemolysis or in equivocal results. The agglutinations were scattered in such a way, however, that no definite groupings comparable to human blood groups could be determined.

Because of non-specific hemolysis, absence of agglutination, or occasional non-specific agglutination, the cell-serum test of Ottenberg proved unreliable in our hands as evidenced by transfusion reactions in previous investigations. The present report concerns the development of a dependable *in vitro* method for demonstrating iso-agglutination in dog blood. The method, which employs principles elaborated by Diamond *et al.* (10) and by Wiener (11), involves the resuspension of saline-washed dog erythrocytes in albumin solution and testing in albumin-diluted dog serum.



## METHOD

*Dogs.* In the selection of dogs animals were purposely included in all states of nutrition and disease, including moribund dogs as well as normal healthy animals. The dogs were mature, represented both sexes, and as a rule were mongrel in type.

*Equipment and Solutions.* All equipment used in the study was chemically clean and was sterilized by heat. The solutions used included sterile 0.85 per cent sodium chloride prepared with redistilled water, bovine albumin solution, and salt-poor human serum albumin readjusted to a salt content of 0.9 to 1.0 per cent.

*Preparation of Erythrocytes.* The following procedure was used: Venous blood was withdrawn without foaming into a chilled saline-wet syringe, one volume of blood being transferred into 6 or 7 volumes of cold saline, centrifuged at 2500 r.p.m., and the supernatant fluid discarded within two minutes of collection of the blood. Two more washings were carried out similarly within four minutes. This rapidity of manipulation was employed 1) in order to keep the temperature as near 4°C. as possible during the entire procedure and 2) in order to avoid having to use anti-coagulants. The above process is really one of differential centrifugation in the cold, the platelets, white cells, and lighter erythrocytes being discarded with the supernatant fluid. The method proved to be highly effective for the preservation of dog erythrocytes. Uniformity of results required, however, that the cells be incubated for a minimum of 15 minutes at 37° to 40°C. and be washed one or more times in warm saline before suspension in albumin solution for the tests of the day.

*Preparation of Serum.* A portion of the blood removed for preparation of cells was transferred to a chilled saline-wet centrifuge tube kept at 4°C. and the serum separated in the cold by centrifugation within about three hours of blood collection. This procedure delayed coagulation of the blood, but it was more effective in the removal of cold auto-agglutinins in one stage than was the procedure of chilling after the clot had formed and partly retracted at room temperature. For routine testing sera were stored at 7°C. for a maximum of about one week.

On the day of testing the serum was diluted with sufficient albumin solution to give a final concentration of 10 to 15 per cent albumin in the cell-albumin-serum mixture.

*Agglutination Test.* The test was conveniently performed by adding one drop of cell suspension (5%) to a mixture of two drops each of serum and of 30 per cent bovine albumin solution in a glass tube (7.0 mm. inside diameter). After mixing, the tubes were centrifuged immediately for 30 seconds at 2500 r.p.m. and the cells resuspended by shaking for the purpose of detecting the presence of any cold auto-agglutinins reacting rapidly at room temperature. The tubes were then incubated for 30 minutes to one hour at 37°C. and after a second brief centrifugation the presence and degree of agglutination were determined after gentle shaking of the warm tubes. Readings were also made at room temperature and after refrigeration at 7°C. over night.

In performing the tests each serum was tested by parallel determinations with autogenous cells and the cells of 5 to 10 other dogs. As the work progressed, the tests were set up by a schedule which permitted each new serum and cell suspension to be tested against the bloods of previously studied dogs, so that agglutinable and

non-agglutinable cells and agglutinating and non-agglutinating sera could be included in the tests of the day to check stability of the cell suspensions and specificity of the agglutinations.

## RESULTS

*In vitro* studies of compatibility of dog blood were made with the erythrocytes of 40 donors and the sera of 36 of these animals. The red cells of 19 dogs were tested with the sera of 25 to 36 dogs; 10 were tested with the sera of 15 to 25 dogs, and 10 were tested with 6 to 15 sera of appropriate type. In addition, multiple cross matching tests were made among 20 of the dogs during an interval of two to nine months. The study involved 1416 individual tests with albumin as a diluent.

Of 40 dogs, 20 had erythrocytes which contained specific agglutinable factors. The agglutinable cells were agglutinated by the sera of dogs having erythrocytes which lacked a specific factor agglutinable by natural dog iso-agglutinins at 32° to 37°C.

*Cross Matching of Bloods of Normal Dogs. Cell-Albumin-Serum Method.* Representative data obtained by the standard procedure using the cells and sera of presumably healthy dogs are illustrated in table 1. Dogs 194 and 413 had received intravenous infusions of pooled or unpooled dog serum three or more months prior to this study, and dogs 696, 628, and 609 had received sera of other dogs by intradermal injection. The results of the tests made with the sera of these animals appeared comparable to results obtained with the sera of other animals and are included in table 1.

On the basis of cross matching among the normal animals, the symbols X, Y, and Z have been used to designate the probable groups into which the bloods could be placed. The blood of one dog (dog 66) contained no iso-agglutinins, and the cells gave weak atypical agglutinations which were poorly sustained at 37°C.

*Other Agglutination Tests.* In 97 tests packed washed red cells were added to undiluted serum. Of 36 anticipated positive reactions, seven results were negative or equivocal at 20° to 24°C. Strong rouleaux formation often complicated the interpretation of weak or negative reactions. Attempts to incubate the mixtures at 37°C. for even 5 to 15 minutes led to non-specific hemolysis in most instances. In general, cells of group Y were agglutinated while the cells of group Z gave poor agglutinations or equivocal tests.

The test using saline solution as a diluent was inadequate in confirming positive results obtained with the other methods. In 42 tests made at room temperature, four positive tests were obtained in 16 anticipated positive reactions. Non-specific hemolysis occurred rapidly at 37°C. The positive reactions were obtained with the cells of group Y, and no false positive agglutinations occurred.

*Cross Matching of Bloods of Ill Dogs with the Bloods of Normal or of Ill Dogs.* Blood samples were obtained from 11 chronically or acutely ill animals, and in 8 of these the bloods were obtained only during illness.

The sera of sick animals gave satisfactory results when tested against known agglutinable or non-agglutinable cells of normal dogs. In the three instances where bloods were also tested prior to illness or after full recovery, the serum prepared during illness gave the results characteristic for that animal.

TABLE I. RESULTS OF CROSS-MATCHING TESTS WITH BLOODS OF NORMAL DOGS<sup>1</sup>

RED CELLS		X SERUM								Y SERUM							Z SERUM			?
Blood group	Dog No.	225	354	609	328	231	353	350	270	628	194	219	413	845	352	169	696	258	244	66
X	225	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	354	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	609	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	328	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	231	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Y	353	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	350	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	270	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
	628	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	194	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Z	219	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	413	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	845	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	352	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	169	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Undeter- mined	696	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	258	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	244	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	66	±	o	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±

<sup>1</sup> The strength of the reactions is indicated by crosses, for instance: + + + + + one large clump, complete agglutination; + — clumps just visible to the naked eye; ± — clumps visible with hand lens. Negative: o — macroscopically homogeneous suspension. The data presented were obtained with varying final serum titers in the range of 1:2 to 1:4.

<sup>2</sup> Agglutination absent at irregular intervals.

Although sera free of non-specific agglutinins were fairly easy to prepare from the bloods of ill dogs, the preparation of cells free of a factor causing agglutination of cells in albumin solution was frequently tedious and time consuming. In general, smooth cell-albumin suspensions could be prepared after the cells had been incubated and thoroughly washed at 43°C. In two instances the cells required washing at 50°C., and satisfactory tests were obtained with the cells so treated, one dog having agglutinable cells and the other non-agglutinable cells by specific reactions. Two typical experiments are illustrated in table 2.

*Miscellaneous Observations.* Inactivation of dog serum (56°C. for 30 min.) frequently intensified its hemolytic action against autogenous or homologous cells and also weakened specific agglutinations. Although dog serum stored for one month may give no non-specific reactions, occasional weak agglutinations at room

TABLE 2. RESULTS OF CROSS-MATCHING TESTS WITH BLOODS OF ILL DOGS AND NORMAL DOGS

BLOOD GROUPING RESULTS BY PREVIOUS TESTS	SERUM  Dog No.	CELLS OF ILL DOGS				CELLS OF NORMAL DOGS					
		Temp. of cell washing				Cells washed at 43°C.			Cells washed at 37°C.		
		37°C.	43°C.	37°C.	43°C.						
		270	270	247	247	219	628	244	66	609	328
X	270	+	o	++++	++++	++++	++++±	++++±	o	o	o
Untested	247			±±	o	o	o	o		o	
Y	219	±±	o	±±	o	o	o	o	o	o	o
Y	628		o		o	o	o	o	o	o <sup>1</sup>	o <sup>1</sup>
Undetermined	66	±±	o		o	o	o	o	o	o	o
X	609	±±	o		+++	++++±	+++++	++++±	±	o	o
X	328	±±	o		++++±	+++±	+++++ <sup>1</sup>	+ <sup>1</sup>	o	o <sup>1</sup>	o

Data illustrating specific and non-specific agglutinations with the bloods of ill dogs. Dog 270 appeared normal until following a large blood donation. Dog 247 exhibited chronic inanition following intestinal resection.

<sup>1</sup> Values were obtained prior to these experiments.

temperature, especially of autogenous and homologous cells, have occurred with sera stored two to four weeks.

For routine tests cells were seldom used after more than two days' storage. However, red cells prepared by cell segregation through selective centrifugation in the cold remained free of spontaneous hemolysis in saline for 6 to 10 days at 7°C. and could usually be agglutinated at room temperature. Such cells in albumin-diluted serum, however, withstood incubation at 37°C. very poorly. The specially prepared cells suspended in albumin were unusually resistant to spontaneous hemolysis even when foamed and left at room temperature for the day or when stored at 7°C. for several weeks.

Although microscopic examinations were not made routinely on negative macroscopic tests, non-agglutinability of the cells of normal dogs in group X and non-agglutinability of cells of group Y by the sera of dogs of group Y was repeatedly confirmed by microscopic examination.

## DISCUSSION

The results of this study confirm the original conclusion of Ottenberg *et al.* (4) that iso-agglutinins occur in the bloods of normal dogs and can be demonstrated in a cell-serum system even though the classical cell-saline-serum method usually gives negative results.

Because of non-specific hemolysis, frequent false negative agglutinations, or auto-agglutinations, the *in vitro* method of Ottenberg *et al.* (4) has proved unreliable in our hands. By using serum albumin solution as a medium for the suspension of specially prepared dog cells and as a diluent for serum previously exhausted of interfering auto-agglutinins active at room temperature or higher, both specificity and increased sensitivity have been obtained with dog blood in macroscopic agglutination tests.

According to current theories conglutination of red cells suspended in serum or albumin solution by an antibody which fails to give visible agglutination when the serum is diluted with saline solution signifies that an antibody of the 'univalent' or

TABLE 3

BLOOD GROUPS		AGGLUTINOGEN IN RED CELLS	ISO-AGGLUTININ IN SERUM	ISO-AGGLUTININ OF INFREQUENT OR IRREGULAR OCCURRENCE
Designation	Incidence			
	%			
X	50	B	$\alpha, \alpha_1$	
Y	40	BA <sub>1</sub>	None	$\alpha_2$
Z	7.5	BA <sub>2</sub>	None	$\alpha_1$ (not observed)
Undetermined	2.5	B (?)	None	?

'blocking' type is present (10, 11). Whether or not this explanation applies to the iso-agglutinations obtained with dog sera is unknown. That inhibition of non-specific hemolysis by albumin was an essential factor in the success of this method is suggested by the observed remarkable power of albumin (10 to 15%) to preserve specially prepared dog cells and to inhibit or delay hemolysis in cell-albumin-serum mixtures during incubation at 37°C. The observations do suggest, however, that the factor adsorbed on certain cells and giving rise to weak agglutination in albumin solution in the absence of serum may be an auto-agglutinin of the 'blocking' type.

The agglutination patterns obtained with the cell-albumin-serum method indicate that the bloods of most mongrel dogs can be placed in one of two major groups. Fifty per cent of the dogs had erythrocytes which were not specifically agglutinable, whereas their sera agglutinated all erythrocytes possessing agglutinable factors. Of the remaining dogs, all had agglutinable cells, and iso-agglutinins were absent except in 4 dogs in which the sera contained weak iso-agglutinins at irregular intervals. Some reluctance was felt in assigning any significance to these inconstant agglutinations of the cells of 3 dogs of group Z by the sera of 4 dogs of group Y. However, in a similar situation, the blood of a dog of group Z (*dog 696*) produced a non-hemolytic reaction on primary transfusion into the recipient. The recipient's response consisted of salivation, severe leukopenia, and delayed disappearance of

bromsulphalein. This incompatibility may be analogous to the *in vivo* incompatibilities encountered occasionally in humans of groups A<sub>1</sub> and A<sub>2</sub>.

According to reports in the older literature the bloods of some dogs absorb anti-A agglutinins, and all dog erythrocytes absorb the human anti-B agglutinin (3, 8). Brockmann (8) in 1911 demonstrated that the sera of some normal dogs specifically agglutinate group A cells of human blood. Data not presented in the present report established the fact that the sera of all the dogs assigned to group X contained agglutinins strongly active against human A<sub>1</sub> and A<sub>1</sub>B cells. Although  $\alpha_1$  agglutinins were lacking in the sera of animals possessing agglutinable cells, the sera of some of the dogs of group Y contained weak anti-O ( $\alpha_2$ ) agglutinins (2).

If all dog erythrocytes contain an agglutinin resembling that of human B cells and if the erythrocytes of certain dogs contain an A-like agglutinin, it appears probable that the latter agglutinin is responsible for the iso-agglutinations found in dog blood. The blood groupings illustrated in table 3 are therefore suggested as being consistent with observations reported in the literature and with those described in this report.

According to this hypothesis and by analogy to the human groups, the two specific dog agglutinogens detected with iso-immune sera by von Dungern and Hirsfeld (7) and designated as A and B by them would resemble those of human A bloods.

#### CONCLUSIONS

1. Dog bloods contain natural iso-agglutinins.
2. The hemolysis occurring in normal dog blood is non-specific.
3. Ninety per cent of mongrel dogs can be placed in one of two major blood groups.
4. A dependable method has been developed for the demonstration of iso-agglutinations in dog blood.

The author wishes to express her appreciation to Doctor M. H. Jacobs for his constant interest and helpful suggestions during the course of this study.

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# MECHANISM OF PROTEINURIA. EFFECT OF PARENTERAL BOVINE ALBUMIN INJECTIONS ON HEMOGLOBIN EXCRETION IN RATS

RICHARD W. LIPPMAN<sup>1</sup>

*From the Department of Medicine, Stanford University School of Medicine*

SAN FRANCISCO, CALIFORNIA

*and the Institute for Medical Research, Cedars of Lebanon Hospital*

LOS ANGELES, CALIFORNIA

**D**URING wartime investigations into the use of substitutes for plasma, it was found by Addis and his associates (1) that massive proteinuria could be induced in rats by the intraperitoneal injection of many proteins. The degree of proteinuria varied with the dose of protein injected and with the specific protein used. Thus, the proteins of small molecular size, such as Bence-Jones protein, appeared in the urine promptly and in large quantities. On the contrary, rat serum, with its homologous protein, required greater dosage and produced less proteinuria.

The mechanism of such induced proteinuria is of considerable interest. While the situation is artificial, from a clinical standpoint, an understanding of its mechanism might indicate directions for investigating the occurrence of proteinuria in human subjects.

Determination of the factors involved in proteinuria raises special difficulties. Such methods as those requiring protein clearance determinations are complicated by difficulties in labelling and identifying specific proteins. The absence of a simple and reliable method for determining albumin, and distinguishing it from other serum proteins, imposes a great handicap. Proteins linked to dye radicals are often toxic, so that they may not be used. Radioactive substances require techniques and facilities that have not been available to us at this time.

This investigation has been based on the use of hemoglobin as an indicator substance. A 7 to 8 per cent purified and non-toxic solution of human hemoglobin (2) was used. This protein is naturally tagged with an identifiable color and with its content of iron.

The excretion of hemoglobin was studied after intravenous injection of various doses in rats, in which a heavy proteinuria had first been induced by intraperitoneal injections of bovine albumin. Under the conditions chosen, protein excretion rates of from 1000 to 1500 mg/24 hrs. are attained during the experimental period. When it is realized that the weight of both kidneys in such a rat is little more than 1000

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Received for publication July 22, 1948.

<sup>1</sup> The author, now at the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, gratefully acknowledges the technical assistance of Way Lew, Bill Wong, and Helen J. Ureen of Stanford University. This work was aided by a grant from the Division of Research Grants and Fellowships, National Institute of Health, U. S. Public Health Service. Bovine albumin (Fraction V) was provided by courtesy of Dr. J. D. Porsche, Armour Laboratories, Chicago, Ill. Hemoglobin was provided by courtesy of Dr. R. B. Pennell, Sharp and Dohme, Inc., Glenolden, Pa.

excretion was much higher than in the control rats, being approximately double at comparable serum hemoglobin concentrations (table 1).

In the dog, Monke and Yuile (5) found a serum concentration threshold at about 100 mg/100 cc., below which hemoglobin did not appear in the urine. Our figure of about 75 mg/100 cc. in the control rats corresponds fairly well with their earlier finding. They estimated the ratio of hemoglobin clearance to glomerular filtration rate in the dog to be 0.03, while in our control animals, using data from other experiments on renal clearances, the ratio was estimated roughly to be 0.04.

Control animals show a slight rise in kidney weight (6%), under the experimental conditions, over the normal weights for rats of the same size on stock diet. The experimental animals, however, show a 22 per cent increase in kidney weight. This change in weight could be due to work hypertrophy and hyperplasia, with an

TABLE 1. EFFECT OF BOVINE ALBUMIN INJECTIONS ON HEMOGLOBIN EXCRETION, AT VARIOUS SERUM HEMOGLOBIN CONCENTRATIONS

SERUM HGB. CONC. AT 2 MIN.	SERUM HGB. CONC. AT 30 MIN.	CALCULATED <sup>1</sup> SERUM HGB. MIDPOINT CONC.	HEMOGLOBIN EXCRETION	HEMOGLOBIN CLEARANCE	BENZIDINE REACTION
Control					
mg/100 cc.	mg/100 cc.	mg/100 cc.	mg/min/G.KWP	cc/min/G.KWP	Most neg.
83	70	77	0.0073	0.00947	
193	147	170	0.0480	0.0282	
791	496	636	0.409	0.0643	
1492	1025	1253	0.619	0.0494	
Experimental					
27	23	25			All strong. pos.
69	61	65	0.0177	0.0272	
186	131	158	0.0669	0.0424	
528	269	386	0.428	0.111	
1066	551	785	0.840	0.107	
1403	634	970	1.134	0.117	

<sup>1</sup> Calculated midpoint assumes that rate of fall in concentration is a log. function of time.

increase in the amount of functioning renal tissue, or it might be due to dilatation of the tubules with fluid and protein, resulting in a non-functional weight increase. Histological studies were performed<sup>2</sup> to distinguish such changes. The tubular lumina of the experimental group contained dense hyaline coagula, representing protein in high concentration. Such coagula, unlike urine, do not escape when the kidney is removed, decapsulated, sectioned, blotted and weighed, and this probably accounts for the weight increase in the kidneys of the experimental group. There was no significant difference in the number of intracellular hemoglobin droplets in the proximal convoluted tubule cells of the control and experimental groups. The urea excretion and urea concentrations, measured in serum and urine after three

<sup>2</sup> Histological examinations were performed by Dr. Lelland J. Rather, Stanford Univ. School of Medicine.



injections of bovine albumin, were no greater than after three injections of sodium chloride solution, so that there did not appear to be any basis for expecting work hypertrophy. Therefore, results were expressed in terms of kidney weight predicted for rats of the same size. However, the differences in hemoglobin excretion between control and experimental groups were so large that they would have appeared significant even though the observed kidney weights had been used.

In view of the threshold shown by normal animals to the excretion of hemoglobin in the urine, it seems likely that, as Yuile has suggested, a given percentage of the hemoglobin molecules presented to the glomerular membrane pass through, and, until the capacity of a reabsorption mechanism is exceeded, do not appear in the urine. Once saturation is achieved, the excretion of hemoglobin becomes a linear function of the serum concentration.

The injections of bovine albumin, by creating an experimental proteinuria, might be expected to saturate the reabsorption mechanism in a non-specific manner. If this were so, the threshold for hemoglobinuria would be lowered, as was observed. However, if that were all, it would then be expected that the line relating excretion to serum concentration in the experimental rats would be parallel to and above the control line. Since the line based on the experimental observations has a much steeper slope than the control line (fig. 2), there must have been an increase in the amount of hemoglobin passing the glomerular membrane.

An increase in the glomerular filtration rate might allow passage of additional quantities of hemoglobin, with no alteration in the glomerular permeability. We have obtained preliminary results which seem to indicate that the difference between inulin clearances determined for the experimental rats and the controls are neither of magnitude nor of direction that would explain the difference in hemoglobin excretion. It seems probable, therefore, that the increase in excretion must be associated with an increase in the glomerular permeability to hemoglobin. Further experiments are in progress, which will attempt to isolate the functions of filtration and reabsorption of hemoglobin.

#### SUMMARY

Intraperitoneal injections of bovine albumin double the excretion of intravenously administered hemoglobin by the rat kidney, at comparable serum hemoglobin concentrations. Under these conditions, the serum concentration threshold at which hemoglobin appears in the urine is lowered from about 75 mg/100 cc. to less than 25 mg/100 cc. It is suggested that the observed results can be explained by the saturation of a protein reabsorption mechanism and an increase in glomerular permeability to hemoglobin.

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# SALYRGAN AND RENAL TUBULAR SECRETION OF PARA-AMINOHIPPURATE IN THE DOG AND MAN<sup>1</sup>

ROBERT W. BERLINER, THOMAS J. KENNEDY, JR. AND JAMES G. HILTON

*From the Research Service, First Division, Goldwater Memorial Hospital and the Department of Medicine, Columbia University, College of Physicians and Surgeons*

NEW YORK CITY

**M**ARKED depression of the tubular secretion of diodrast and para-aminohippurate (PAH) following the injection of salyrgan (mersalyl)<sup>2</sup> in human subjects has been reported by Brun, Hilden, and Raaschou (1). An earlier observation in this laboratory had indicated that this function in the dog was not impaired during mercurial diuresis. The discrepancy seemed to warrant further investigation.

## METHODS

Animal experiments were performed on trained, unanesthetized female dogs. Observations in man were obtained using patients with no evidence of renal disease.

Inulin in patients, creatinine in dogs and PAH in both were administered by continuous intravenous infusion. All urine collections were made with an indwelling catheter and each clearance period was terminated by washing the bladder with distilled water. Heparinized venous blood samples were obtained at the midpoint of each clearance period. The periods were of 10 to 20 minutes duration depending on the urine flow.

Creatinine was determined in tungstic acid filtrates of plasma and in diluted urine by a modification of the Folin method (2). Inulin was determined in zinc filtrates of plasma and in diluted urine by the method of Harrison (3). Glucose was removed from plasma by treatment with yeast before precipitation. PAH was determined in trichloroacetic acid filtrates of plasma and in diluted urine by the method of Bratton and Marshall (4). Sodium was determined in urine and plasma by internal standard flame photometry (5) with an error of less than one per cent. Chloride was determined by a modified Volhard titration (6).

The secreted PAH ( $T_{PAH}$ ) was calculated as the difference between excreted and filtered PAH. In the calculation of the filtered PAH, 83 per cent of the plasma PAH was assumed to be free in the plasma water (7). Since marked disturbances of plasma proteins were not present in the experimental subjects and since interest centered upon changes in transfer capacity ( $T_{M_{PAH}}$ ), rather than on its absolute value, the use of this empirical factor in both dog and man does not significantly influence the

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Received for publication July 22, 1948.

<sup>1</sup> This investigation was supported in part by grants provided by the National Institute of Health (U. S. P. H. S.) and the Josiah Macy, Jr. Foundation.

<sup>2</sup> The salyrgan used in this study was supplied by the medical research department of Winthrop-Stearns, Inc.

results. Loads presented to the tubule for secretion were calculated on the assumption of a filtrate fraction of 0.20 in man and 0.30 in the dog.

The pattern of all experiments was similar. Each was initiated with a priming dose of PAH and inulin (or creatinine). After 20 to 40 minutes for equilibration two or three control urine collection periods were obtained. A dose of salyrgan, 2 ml. in patients, one ml. in dogs (or 4 to 6 mg.  $\text{HgCl}_2$  in some dog experiments) was then administered intravenously. An interval of 30 to 45 minutes was allowed for the mercurial to exert its effect before the three or more post-mercurial urine collection

TABLE 1. EFFECT OF SALYRGAN ON  $\text{TM}_{\text{PAH}}$  IN THE DOG (EACH FIGURE IS THE AVERAGE OF 3 CLEARANCE PERIODS)

DOG	BEFORE SALYRGAN		AFTER SALYRGAN			AVERAGE RATIO, LOAD/T
	$\text{T}_{\text{PAH}}$	Sodium excretion	$\text{T}_{\text{PAH}}$	$\Delta\text{T}_{\text{PAH}}$	Sodium excretion	
	mg/min.	$\mu\text{eq/min.}$	mg/min.	%	$\mu\text{eq/min.}$	
B	30.1	93	32.1	+6.7	415	1.8
E	12.8	162	12.5	-2.3	822	5.5
F	17.4	136	17.5	+0.6	863	2.0
M	12.4	260	10.7	-13.7	1652 <sup>1</sup>	5.0

<sup>1</sup> Within 30 min. of the injection of BAL (3 mg/kilo) the sodium excretion and urine flow were reduced below control levels without change in the  $\text{TM}_{\text{PAH}}$ .

TABLE 2. EFFECT OF SALYRGAN ON  $\text{TM}_{\text{PAH}}$  IN MAN (EACH FIGURE IS THE AVERAGE OF 2 TO 3 CLEARANCE PERIODS)

PATIENT	BEFORE SALYRGAN				AFTER SALYRGAN				
	$\text{T}_{\text{PAH}}$	Load/T	Chloride excretion	Sodium excretion	$\text{T}_{\text{PAH}}$	Load/T	$\Delta\text{T}_{\text{PAH}}$	Chloride excretion	Sodium excretion
	mg/min.		$\mu\text{eq/min.}$	$\mu\text{eq/min.}$	mg/min.		per cent	$\mu\text{eq/min.}$	$\mu\text{eq/min.}$
H	106	1.0	149	692	34.5	5.0	-67	382	867
L	67.5	1.0	12	286	16.4	6.2	-76	258	368
M	71.5	1.6	204	617	13.7	11.0	-81	297	532
P	71.0	1.9	233	663	17.5	13.0	-75	475	890
T	113	1.5	47	409	19.7	18.0	-83	1415	1292

periods were obtained. In some experiments a dose of 2,3 dimercaptopropanol (BAL) was then administered intramuscularly and several additional urines collected.

## RESULTS

The effect of salyrgan on  $\text{TM}_{\text{PAH}}$  was observed in four experiments on 4 dogs. The results are summarized in table 1. In no instance was there any significant change in the  $\text{TM}_{\text{PAH}}$ , despite the striking increase in sodium excretion and the usual diuresis.

A sharp difference is apparent in man. The results of experiments in 5 normal subjects are presented in table 2. In each experiment there was a marked depression of the secretion of PAH, ranging from 67 to 83 per cent. In several experiments (subjects H and L) the load presented to the tubules for secretion in the presalyrgan

periods was probably too small to insure saturation of the mechanism. The  $T_{PAH}$  in these patients was probably higher than that observed and the depression of the secretory capacity greater than indicated by the figures obtained.

The protocol of the experiment on *patient T* is presented in detail in table 3. The injection of salyrgan depressed the  $T_{PAH}$  by about 80 per cent. Following the administration of 5 mg./kilo of BAL, the effect of the diuretic on electrolyte excretion

TABLE 3. PROTOCOL OF EXPERIMENT ON PATIENT T SHOWING EFFECT OF SALYRGAN AND OF BAL ON TUBULAR SECRETION OF PAH

TIME	URINE FLOW	INULIN CLEARANCE	SODIUM EXCRETION	CHLORIDE EXCRETION	PLASMA PAH	$T_{PAH}$
min.	ml/min.	ml/min.	$\mu\text{eq/min.}$	$\mu\text{eq/min.}$	mg. %	
0	Complete injection of priming inulin 3.75 gm., PAH 6 gm.					
1	Start infusion of inulin 0.9%, PAH 3.2% in normal saline at 5 ml/min.					
25-46	2.85	147	424	61	27.7	109
46-69	2.86	151	420	50	24.8	120
69-92	2.42 <sup>1</sup>	132	382	31	24.0	110
93	Salyrgan 2 ml. i.v.					
139-155	4.16	124	561	524	50.7	10.6
155-164	12.32	146	1755	1940	56.1	22.0
166	BAL 5 mg./kilo i.m.					
164-176	10.88	115	1560	1780	60.3	25.9
176-195	3.46	136	576	40	54.5	107
195-215	2.78 <sup>1</sup>	112	461	31	50.1	98

<sup>1</sup> Values of all excretion rates in these periods are probably low because of incomplete urine collection. The increases required to bring the inulin excretion up to the av. of the remaining periods are 14 and 12% respectively.

TABLE 4. EFFECT OF  $\text{HgCl}_2$  ON  $T_{PAH}$  IN THE DOG

DOG	BEFORE $\text{HgCl}_2$		AFTER $\text{HgCl}_2$			AFTER BAL		AVERAGE RATIO, LOAD/T
	$T_{PAH}$	Sodium excretion	$T_{PAH}$	$\Delta T_{PAH}$	Sodium excretion	$T_{PAH}$	Sodium excretion	
	mg/min.	$\mu\text{eq/min.}$	mg/min.	%	$\mu\text{eq/min.}$	mg/min.	$\mu\text{eq/min.}$	
B	13.0	157	14.7	+13.1	384	12.8	294	6
E	17.4	272	18.3	+5.2	403	18.0	186	2
L	11.5	445	11.9	+3.5	733	13.5	483	11
M	10.9	31	9.3	-14.7	491	9.6	244	6

was very rapidly dissipated and the  $T_{PAH}$  was restored almost to the control value. In two other experiments, smaller doses (up to 2.5 mg./kilo) of BAL did not have any definite effect on either electrolyte excretion or  $T_{PAH}$ . It should be noted that the control sodium excretion in these experiments is high because of the cation required to cover the PAH. The effect of salyrgan is to reduce the PAH excretion sharply and, with this, to reduce the cation excretion necessitated by the excretion of PAH. For this reason, the changes in chloride excretion are more striking than those in sodium and the chloride has been included in the summary table 2 as an indication of the diuretic effect of the salyrgan.

It seemed important to determine whether the difference between man and dog, in the response of the  $T_{M_{PAH}}$  to salyrgan, was attributable to differences in the susceptibility of the transfer mechanism to inhibition by mercury or to differences in the handling of the organic salyrgan molecule. Experiments were therefore performed on 4 dogs in which small doses of mercuric chloride were injected intravenously. These doses were sufficient to produce diuresis without, presumably, inducing severe renal damage and were of the same order of magnitude as those used in man by Sollman, Schreiber, and Cole (8) in a study of the diuretic effect of a number of mercury compounds. The effects on sodium excretion and on the  $T_{M_{PAH}}$  are presented in table 4. The diuretic effect of  $HgCl_2$ , although definite, was less striking in these experiments than that of salyrgan. As in the salyrgan experiments, there was no appreciable depression of the  $T_{M_{PAH}}$ . Injection of a single dose of approximately 5 mg/kilo of BAL depressed the excretion of sodium and chloride towards the control values. The interruption of the diuresis was not, however, as dramatic as when the diuresis had been induced with salyrgan.

The difference between control  $T_M$  values observed in 2 of the dogs, *B* and *E*, which are the same in tables 1 and 4, warrants comment. The control values of  $T_{M_{PAH}}$  in *dog B* indicate a fall of 57 per cent between the first and second experiments, an interval of two months. On the other hand, the  $T_{M_{PAH}}$  in *dog E* rose 36 per cent in the five-month interval between the two experiments. No ready explanation for these changes is apparent. Stability from month to month of the transfer capacity for PAH in the dog has, however, never been established.

#### DISCUSSION

The fact that the renal tubular capacity to transfer PAH in man is markedly depressed by salyrgan while in the dog it is unaffected indicates a fundamental difference in the transfer mechanism. It had long been postulated that the action of the organic mercurial diuretics is due to the liberation of small amounts of mercury ion from the organic complex (8). This is supported by the effect of BAL in completely reversing the effects of the organic mercurials on both electrolyte reabsorption and PAH secretion. Failure to depress the  $T_{M_{PAH}}$  in the dog does not seem attributable to a difference in the handling of the salyrgan itself. In the dog, the effect of salyrgan on electrolyte reabsorption does not differ from that observed in man. Its reversal by BAL is rapid and complete (9). Furthermore, the diuretic effect can be produced by the injection of ionic mercury without affecting the transfer of PAH.

One point of difference between dog and man in the transfer mechanism for PAH has previously been noted, i.e. the same mechanism appears to be involved in the secretion of creatinine in man (10), whereas no secretion of creatinine occurs in the dog. This may not be germane to the difference in response to mercury since salyrgan has been reported to depress the diodrast  $T_M$  of the rat (11), a species in which creatinine secretion does not occur (12).

The data reported re-emphasize the need for caution in applying information concerning tubular transport mechanisms in one species to another.

## SUMMARY

The  $T_{M_{PAH}}$  of man is markedly depressed by salyrgan. This depression, as well as that of electrolyte reabsorption, is reversed by BAL. Salyrgan and small doses of  $HgCl_2$  do not depress the  $T_{M_{PAH}}$  in the dog, indicating a significant difference in the tubular mechanism for transferring PAH.

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# RENAL GLUTAMINASE<sup>1</sup>

E. MYLON AND J. H. HELLER<sup>2</sup>

*From the Laboratory of Pathology, Yale University School of Medicine*

NEW HAVEN, CONNECTICUT

THE presence of significant amounts of glutamine in various animal tissues has been established (1, 2). Studies in recent years with labelled ammonia-nitrogen (3) have shown that glutamine accepts as well as contributes ammonia, providing an important means for the detoxification, storage and transportation of this base. It has also been learned that the introduction of ammonia into glutamic acid as well as the reverse process, the hydrolytic liberation of ammonia from glutamine, is catalyzed by the enzyme glutaminase (4). The hydrolytic action of glutaminase is dominant in the kidney while in other tissues the synthetic action of the enzyme seems to prevail (4, 5). It is not known whether this difference is based on different types of glutaminase or whether a shift in the equilibrium of the enzymatic reaction is caused by special activators or inhibitors operating in different tissues. The formation of urinary ammonia and its manifold increase in acidosis has been explained by glutamine hydrolysis (6). The suggestion that the stimulus for increased ammonia formation in acidosis might be related to the lowering of the  $pH$  of the plasma or the decline of its bicarbonate concentration (7) should be enlarged to include formation or mobilization of specific activators for renal glutaminase. The question arises whether under pathological conditions the equilibrium of glutaminase action in other tissues might change in the direction of hydrolysis. Were this to occur, the ammonia formed might well prove toxic at its site of formation. Studies were initiated to explore this possibility and also the presence of activators and inhibitors of hydrolysis or synthesis of glutamine. The report that follows is concerned with studies on kidney glutaminase and especially with the activation of the enzyme by phosphate ions and beta-hydroxybutyric acid.

## MATERIALS AND METHODS

Freshly prepared tissue slices were used as enzyme preparation of glutaminase. Immediately following extirpation of a dog or cat kidney, the organ was speedily frozen by aid of solid  $CO_2$  and kept in a deep freezer until used. For each determination the frozen kidney was placed on a small block of solid  $CO_2$ , very thin slices were shaved from the cortex with a razor blade, rapidly weighed and placed in a test tube containing 3 cc. of buffer and either 2 cc. of saline (blank) or 2 cc. of freshly prepared glutamine solution, containing 1 mg. of glutamine per cc.<sup>3</sup>

Received for publication July 7, 1948.

<sup>1</sup> Aided by a Grant from the Commonwealth Fund.

<sup>2</sup> Fellow of the Dazian Foundation for Medical Research.

<sup>3</sup> We are indebted to Dr. H. V. Vickery for the supply of purified glutamine.

After incubating the tubes in a water bath at  $37.5^{\circ}\text{C}$ . for varying lengths of time glutaminase action was stopped by lowering the  $p\text{H}$  to 5.0 through the addition of 1 cc. of 1 N sulfuric acid.

After deproteinization by addition of 1 cc. of 10 per cent sodium tungstate solution and 1 cc. of  $\frac{2}{3}$  N sulfuric acid, the tubes were centrifuged and an aliquot of the protein-free supernatant, made up to 5 cc. with distilled water, was placed in an Evelyn colorimeter. Five cc. of Nessler's solution prepared according to Folin-Wu were added and the content of the tubes mixed. The tubes then were read as soon as possible with a 440  $m\mu$  filter.

The rapid development of turbidity in many tubes, particularly in the glutamine-free blanks, was a vexing problem, necessitating both the use of diluted aliquots and rapid reading. To test the accuracy of the values, direct Nesslerization was checked by both the permutit and the aeration method. In both these procedures, the initial steps for the determination of ammonia were identical including the precipitation of the proteins and centrifugation mentioned above. Then 1 gram of permutit was added to an aliquot of the supernatant, the tube shaken for 30 seconds and the permutit centrifuged off. The ammonia was eluted from the permutit with 10 cc. of  $\frac{1}{10}$  N HCl solution in 3 per cent NaCl, and after the permutit was centrifuged off, an aliquot of the supernatant was used for Nesslerization. For the aeration method a slightly modified Van Slyke urea apparatus (9) was used. Phosphate buffers were made up of  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  to the various  $p\text{H}$ 's and molarities mentioned below. Veronal buffers were made up to  $m/20$  and the  $p\text{H}$  was adjusted with  $n/10$  HCl or  $n/10$  NaOH.

To contrast tissue slice with organ extract preparations, kidney cortex was ground with sea sand or homogenized with a Ten Broeck (10) apparatus. One cc. of the homogenate, corresponding to 800 mg. of kidney cortex was diluted as indicated below. The liberated ammonia in these experiments was determined both by direct Nesslerization and with the aeration method. Pyruvic acid used in these experiments was obtained from the Eastman Kodak Company, Rochester, New York, and beta-hydroxybutyric acid, from the Paragon Testing Laboratories, Orange, New Jersey<sup>4</sup>.

## RESULTS

*Ammonia Formation in Tissue Slices in the Absence of Added Glutamine.* Early in the course of these studies it was observed that ammonia formation in tissue slices prepared as described above is rather small. This is a favorable contrast to the disturbing ammonia production in extracts and homogenates that require special precautions, including prolonged dialysis at  $0^{\circ}\text{C}$ . as well as addition of potassium cyanide (5). As will be shown later glutaminase activity is so much greater in tissue slices than in homogenates or extracts that a few minutes of incubation suffice. This helps still further to reduce ammonia formation in controls.

*pH Optimum of Glutaminase.* pH series were prepared with  $m/20$  veronal buffer. The maximal glutaminase activity in three sets of experiments was found to be at or

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<sup>4</sup> Kindly supplied by Dr. A. E. Wilhelmi.



near pH 7.8. Figure 1 is representative of the three experiments and indicates the dependency of glutaminase activity on hydrogen ion concentration (4).

Control experiments were carried out in which veronal was replaced by different amounts of  $n/100$  NaOH. Since the pH despite the lack of any buffer solution did not change more than 0.2, direct comparison with the buffered solution was possible. It was found that the glutaminase activity was uninfluenced by the addition of  $m/20$  veronal.

*Enzyme Amount.* The influence on glutamine hydrolysis of different enzyme amounts was studied in 14 series of experiments. The weights of the tissue slices served in lieu of volume amounts. Results were rather uniform and are represented by the following example: Liberation of  $\text{NH}_3\text{-N}$  from 2 mg. of glutamine on 10 minute

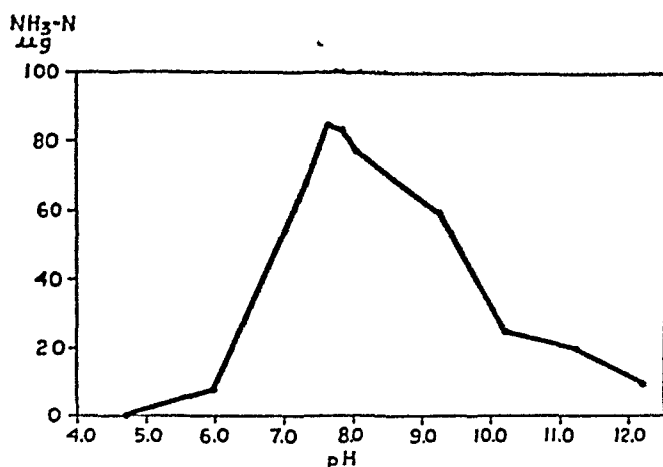
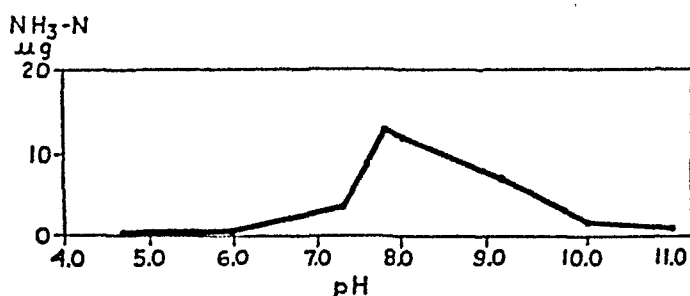


Fig. 1 (upper). pH CURVE OF RENAL GLUTAMINASE IN VERONAL BUFFER.

Fig. 2 (lower). pH CURVE OF RENAL GLUTAMINASE IN PHOSPHATE BUFFER.

incubation with tissue slices of 400, 150, and 80 mg. wet weight was found to be 163, 92, and 43  $\mu\text{g}$ . respectively, corresponding to an hydrolysis of 85, 48, and 22 per cent. Tissue slices of approximately 80 mg. wet weight were found to be convenient for these experiments and henceforth were used.

*Enzyme Activity in Tissue Slices as Compared to Enzyme Activity in Extracts.* The relatively low glutaminase activity of renal extracts or homogenates observed early in these studies was one of the reasons of utilizing tissue slices. Many experiments were carried out to compare quantitatively the activity of slices with extracts and homogenates. One example follows: homogenates containing 800, 400,

and 80 mg. of cortex, liberated on 10-minute incubation 122.3, 60.8, and 13.2  $\mu$ g. of  $\text{NH}_3\text{-N}$  from 2 mg. of glutamine while a cortex slice of 80 mg. liberated under identical conditions of substrate, temperature,  $\text{pH}$  and time 63.2  $\mu$ g. of  $\text{NH}_3\text{-N}$ . It can be seen that weight per weight the activity of glutaminase in tissue slices is more than five times as great as that of tissue homogenates.

*Substrate Amount and Incubation Time.* The amounts of glutamine as well as the incubation times were varied in three sets of experiments. Table 1 is representative of one of these.

It should be mentioned that each set of experiments was carried out on slices of one individual kidney cortex. This precaution was taken after it had been observed that the glutaminase activity of kidney cortices of different dogs may vary considerably.

*Activators of Glutaminase: A. PHOSPHATE.* Replacement of veronal buffers with phosphate buffers left the  $\text{pH}$  optimum unchanged, i.e. near 7.8. Figure 2 represents the result of a typical experiment.

Another fact became evident in these experiments. Comparison between the curves obtained with veronal and with phosphate buffer confirmed previously reported activation of glutaminase by phosphate anions (11-13). However, the full

TABLE 1. INFLUENCE OF THE SUBSTRATE CONCENTRATION AND OF THE INCUBATION TIME ON THE ACTION OF RENAL GLUTAMINASE

GLUTAMINE mg.	INCUBATION TIME min.	$\text{NH}_3\text{-N}$ HYDRO- LYZED BY GLUTAMINASE $\mu$ g.	GLUTAMINE mg.	INCUBATION TIME min.	$\text{NH}_3\text{-N}$ HYDRO- LYZED BY GLUTAMINASE $\mu$ g.	GLUTAMINE mg.	INCUBATION TIME min.	$\text{NH}_3\text{-N}$ HYDRO- LYZED BY GLUTAMINASE $\mu$ g.
0.4	3	1	0.4	6	6	0.4	10	7
2.0		5	2.0		12	2.0		34.5
6.0		25	6.0		40	6.0		59

degree of this activation seems to be recognizable only by the use of the tissue slice method. The following example is representative for 6 different experiments carried out identically: liberation of  $\text{NH}_3\text{-N}$  from 2 mg. of glutamine without buffer ( $\text{pH}$  7.8), 8.0  $\mu$ g., with veronal buffer ( $\text{pH}$  7.8) 8.5  $\mu$ g. and with phosphate buffer ( $\frac{m}{3}$ ,  $\text{pH}$  7.8) 64.0  $\mu$ g.

*Optimum Concentration of Phosphate for Activation of Glutaminase.* Table 2 shows the influence of variation of phosphate concentration on the glutaminase activity.

As can be seen the optimal final concentration of phosphate was found to be between 1/4 and 1/6 molar. However even 1/30 molar final concentration activates glutaminase greatly.

*B. PYRUVATE.* A second acid has been tested in several tissue slice experiments. In accordance with previous observations (11-13), activation of glutaminase with pyruvate was marked. Nevertheless the action of pyruvate was significantly less than that of phosphate. When these two activators were combined they did not exhibit a cumulative effect; on the contrary, in several experiments of this kind the activation of this combination was slightly less than that of the phosphate alone.

C. BETA-HYDROXYBUTYRIC ACID. In the search for other activators of renal glutaminase, attention was directed to the circumstances existing in diabetic acidosis. On the basis of the observation that glutamine is the chief precursor of urinary ammonia (6) the large increase of the latter substance in acidosis may be referred to an increased glutamine hydrolysis. It seemed, therefore, of interest to study the influence of beta-hydroxybutyric acid on the activity of renal glutaminase. In a series of 70 experiments carried out in the course of these studies it was found that beta-

TABLE 2. INFLUENCE OF INORGANIC PHOSPHATE ON THE ACTIVITY OF RENAL GLUTAMINASE

MOLARITY OF PHOSPHATE BUFFER <sup>1</sup>	NH <sub>3</sub> -N LIBERATED	WEIGHT OF SLICES
	μg.	mg.
1	85.1	80
1/2	97.9	
1/3	97.4	
1/5	76.6	
1/15	77.3	
1/50	13.5	
1/100	10.7	

<sup>1</sup> 3 cc. used in 5 cc. total volume, pH 7.8.

TABLE 3. ACTIVITY OF GLUTAMINASE IN VERONAL BUFFER, IN PHOSPHATE BUFFER AND IN PHOSPHATE BUFFER PLUS BETA-HYDROXYBUTYRIC ACID

EXP. NO.	NO BUFFER pH 7.8	VERONAL m/20	PHOSPHATE	PHOSPHATE + 1 MMOL. β-HYDROXY- BUTYRIC ACID
	NH <sub>3</sub> -N μg.	NH <sub>3</sub> -N μg.	NH <sub>3</sub> -N μg.	NH <sub>3</sub> -N μg.
1	8.5	8.0	64	141.1

TABLE 4. ACTIVATION OF RENAL GLUTAMINASE BY BETA-HYDROXYBUTYRIC ACID

EXP. NO.	PHOSPHATE BUFFER	PHOSPHATE BUFFER + 1 MMOL. β-HYDROXYBUTYRIC ACID
	NH <sub>3</sub> -N μg.	NH <sub>3</sub> -N μg.
2	50.0	90
3	18.3	62.4
4	38.1	109.3

hydroxybutyric acid activates glutaminase in veronal buffer to about the same extent as pyruvate, while in phosphate buffer it activates the enzyme to an exceedingly high degree.

Tables 3 and 4 contain the results of four representative experiments.

A fifth experiment indicating the great differences in renal glutaminase activity in veronal buffer, phosphate buffer and phosphate buffer plus beta-hydroxybutyric acid is illustrated in figure 3.

*Influence of the Amount of Beta-hydroxybutyric Acid.* In the six series of experiments increase of beta-hydroxybutyric acid from 0.5 to 1.0 millimole was associated

with a progressive increase in the enzyme activation. In one representative experiment liberation of  $\text{NH}_3\text{-N}$  on addition of 0.5 millimole, 1.0 millimole, and 2.0 millimole of beta-hydroxybutyric acid was found to be 66.1, 76.4, and 97.9  $\mu\text{g}$ . Higher concentrations have not as yet been tested.

D. LACTIC AND ACETIC ACID. When beta-hydroxybutyric acid in veronal buffer was replaced by the same concentration of either lactic or acetic acid only insignificant activation of glutaminase was observed. When, however, these acids were used in combination with phosphate buffer there was a marked inhibition of the activating action of phosphate. Table 5 contains the results of a representative experiment of the series of 12.

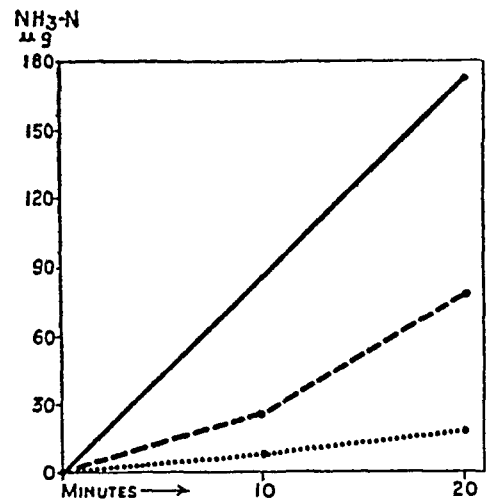


Fig. 3. RENAL GLUTAMINASE ACTIVITY in veronal buffer ( . . . ), in phosphate buffer ( - - - - ) and in phosphate buffer plus 2 millimole of beta-hydroxybutyric acid ( — ).

TABLE 5. ACTIVATING AND INHIBITING INFLUENCES ON RENAL GLUTAMINASE

VERONAL M/20	PHOSPHATE M/3	PHOSPHATE + $\beta$ -HYDROXYBUTYRIC ACID (1 MMOL.)	M/3 PHOSPHATE + LACTIC ACID (1 MMOL.)	M/3 PHOSPHATE + ACETIC ACID (1 MMOL.)
$\text{NH}_3\text{-N } \mu\text{g.}$	$\text{NH}_3\text{-N } \mu.$	$\text{NH}_3\text{-N } \mu\text{g.}$	$\text{NH}_3\text{-N } \mu\text{g.}$	$\text{NH}_3\text{-N } \mu\text{g.}$
10.0	42	96	24	33

DISCUSSION

The application of the tissue slice method for the determination of renal glutaminase proved to be advantageous. The enzymatic activity in slices prepared from renal cortex was significantly greater than that in extracts or homogenates of the same organ. The high activity of the enzyme permitted reduction of the incubation time to a few minutes. During this short incubation production of ammonia in the glutamine free solution (blank) was very small. The activating influence of inorganic phosphate and pyruvate, previously reported for extracts (11, 12) was also demonstrated in slices of kidney cortex. The observation that beta-hydroxybutyric acid in combination with inorganic phosphate is the strongest activator for renal glutaminase as yet observed is remarkable in the light of the conditions prevailing both in diabetic acidosis and in hyperglycemia. Increased production of betahydroxybutyric acid in diabetes is known to be associated with an increased formation of

urinary ammonia, and alimentary hyperglycemia has been recently reported to be followed by an elevated phosphatase action in renal tubules, i.e. increased liberation of inorganic phosphates (14, 15). Whether the *in vitro* results on phosphate and beta-hydroxybutyric acid suffice to explain the mechanism of increased urinary ammonia formation requires further investigation.

#### SUMMARY

The activity of renal glutaminase in tissue slices prepared from frozen kidney cortex is significantly greater than that in extracts or homogenates of the same organ. The stimulation of the enzymatic activity in tissue slices by pyruvate and phosphate is marked.

The greatest stimulation of renal glutaminase was obtained by a combination of phosphate and beta-hydroxybutyric acid. Possible relationship of this combination to the increased formation of urinary ammonia in diabetic acidosis is suggested.

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## CORRIGENDA

*Volume 151, page 30, lines 4 and 6.* The dissociation constant should read  $2 \times 10^{-6}$  instead of  $2 \times 10^{-3}$ .

*Volume 152, page 340.* Under table 2, top, right-hand column, "coronary, mean  $\pm \sigma_m$ ", the first entry should read:  $13.0 \pm .54$  instead of  $13.0 \pm 5.4$ .

*Volume 154, page 37.* Insert the following paragraph before SUMMARY AND CONCLUSIONS:

Finally, in connection with a discussion of T-1824 clearance, it is interesting and informative to note the tabulated data of electrophoretic studies on T-1824 and protein in urine of human nephrosis (17). Although it is not stated how dye was measured in nephrotic urine, the T-1824 clearance was 0.37 ml. per minute per square meter of body surface, the albumin clearance was 0.39 while total protein clearance was 0.09. The globulin clearance of 0.03 was very small indeed. Luetscher (17) preferred in 1944 to compare dye with total protein clearance, but since the clearances for T-1824 and serum albumin were nearly equal and considerably greater than that of globulin it is probable that the dye was excreted in the form of dye albumin. In nephrosis, then, the kidney behaves as though injected dye combined selectively with serum albumin leaving vanishingly small amounts of free dye in the blood. On the basis of body surface area a human nephrotic can have a dye clearance 100 times greater than that of a normal dog. This again suggests that dye clearance when analyzed by a sensitive method is a measure of normally occurring albumin clearance.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME 155

*October-December 1948*

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Washington, D. C.



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*Made in United States of America*

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

Published by  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 155

October 1, 1948

NUMBER 1

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## ELECTROPHRENIC RESPIRATION<sup>1</sup>

STANLEY J. SARNOFF, ESTHER HARDENBERGH AND  
JAMES L. WHITTENBERGER

*From the Department of Physiology, Harvard School of Public Health*

BOSTON, MASSACHUSETTS

**P**RESENT methods of artificial respiration, with the exception of the body-enclosing respirators (1, 2), introduce unphysiologic factors which, under various circumstances and for various reasons, are undesirable. Beecher, Bennett and Bassett (3) have recently examined the adverse effects of elevated intratracheal pressure on the circulation in 'shock' states. A detailed critique of the various methods of artificial respiration will not be presented here. Suffice it to say that, if the diaphragm could be made to contract and relax in a controllable manner, a method of artificial respiration more nearly simulating natural breathing would be available. Such a method would obviate the necessity for the expense and bulk of the body respirator.

It has long been known that the response of a muscle to the stimulation of its nerve is in direct proportion to the intensity of the applied stimulus (4, 5). Theoretically, therefore, a continuously varying, undulating stimulus applied to a nerve should result in a continuously varying, undulating contraction of the innervated muscle. Since an electrical stimulus applied to a nerve can be readily varied in contour and rate, the production of artificial respiration by appropriate stimulation of the phrenic nerve seemed a logical possibility. This report describes the application of such a technique to the production of artificial respiration in several species of laboratory animals.

### METHOD

The experiments were performed on the cat, dog, monkey and rabbit. The anesthesia used is indicated in table 1. Barbiturates were, for the most part, not used because of the difficulty of avoiding some degree of respiratory depression. In one experiment (no. 17) nembutal was used for

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Received for publication August 5, 1948.

<sup>1</sup> Aided by a grant from The National Foundation for Infantile Paralysis.

anesthesia and respiratory depression was minimized by the use of small doses. Nembutal was used in large doses in the one experiment (no. 18) in which it was desired to produce respiratory depression due to barbiturate poisoning.

Blood samples were collected under oil or over mercury and analyzed immediately for  $O_2$  and  $CO_2$  tensions according to the method of Riley (6). Determinations were done in duplicate. Minute volumes were obtained by collecting expired air in a spirometer.

An electrode (or, in some cases, two electrodes) was brought into contact with the main trunk of the phrenic nerve low in the neck posterior to the subclavian vein, at which point the phrenic filaments from the various levels of the cervical outflow join together or lie in close proximity (fig. 1). After incision of the skin, a cleavage plane was developed between the external jugular vein and the sternocleidomastoid muscle; the latter was retracted medially exposing the uppermost filament of the phrenic nerve in its position on the posterior cervical fascia. This filament lies lateral to and parallel to the carotid sheath; it was followed downward and the point found at which the phrenic filaments converged. Each filament was tested electrically to be sure of its function. Proper electrode implantation was most easily accomplished in the dog, next most easily in the cat. It was difficult to accomplish proper electrode application in the monkey because of the very short neck, high clavicle and diffuse outflow that makes up the phrenic bundle. The latter forms a single trunk quite low in the neck, an arrangement very different from that found in man. Rabbits were used in the preliminary experiments and although it is feasible to produce electrophrenic respiration in that species, the delicacy of the nerve, the low blood volume and the difficulty of doing repeated arterial punctures make it unsuitable for experiments involving blood gas analysis. The difficulty of abolishing respiration is likewise greater in the rabbit.

Several different ways of making and implanting the electrode were tried. The following is the best method developed thus far. Four no. 34, pure silver strands were wound spirally until they formed a fine, multiple-strand wire; this was cut into 12-inch lengths, each to serve as both electrode and lead wire. Each unit was covered snugly with polyethylene tubing (free of plasticizer) with an inside diameter of 0.34 inch and an outside diameter of 0.48 inch. Three quarters of an inch of the wire at each end was left bare, one end to serve as the stimulating electrode, the other to be connected to the source of current. A flat piece of pure polyethylene sheeting (free of plasticizer), 2 inches by  $\frac{3}{4}$  inch by 0.0015 inch, was placed under the phrenic trunk (fig. 1). One bared end of the silver wire was placed around the nerve and twisted on itself so that the phrenic nerve could not escape contact with the electrode in some portion of its perimeter. A silk suture was used to secure the distal to the proximal limb of the loop. An additional silk suture then secured the insulating sheeting to the electrode and to itself. In this way the phrenic nerve could not escape contact with the electrode but was not compressed by it and the latter could not deliver an impulse to any other structure. The other end of the silver wire was threaded onto a surgical needle and led out through the skin at some distance from the original wound. The wound was then closed. In order to complete the circuit, an indifferent electrode in the form of a hypodermic needle or ECG plate was applied at some convenient distant point on the body.

This arrangement was found to be most satisfactory for several reasons:

- 1) The loop lies loosely about the nerve and does not compress it although it is always in contact with it.

- 2) Having a single electrode in contact with the nerve avoids the occurrence of the possible ischemia in that segment of nerve which would otherwise lie between two electrodes.

- 3) The fine lead wire can be threaded onto a surgical needle and made to emerge from the neck at a considerable distance from the incision so that the latter can be closed. Using only one electrode instead of two diminishes whatever undesirable effects may attend the purposeful implantation of a foreign body.

- 4) If both phrenic nerves are stimulated, the electrode on one nerve serves as the indifferent electrode for the opposite side and vice versa so that the externally applied electrode can be dispensed with; indeed, two electrodes on each nerve would be superfluous under those conditions.

- 5) Polyethylene is a good insulator for both the silver wire and nerve because of its demonstrated inertness in contact with nervous tissue (7).

The current used for nerve stimulation was delivered by a Grass Stimulator, although a much

simpler and less expensive device could be used, now that the limits of frequency, duration and voltage are established. Individual stimuli having a duration of 2 milliseconds and delivered at a frequency of 40 per second were found suitable for phrenic nerve stimulation. The current was fed through a rotating potentiometer which delivered a voltage that varied regularly between 0 and about 3 volts. Such a method of stimulation makes the diaphragm perform a reasonable imitation of its normal respiratory motion. The rate at which the lever arrangement actuates the rotating potentiometer can be varied from 0 to 60 per minute either by means of a friction clutch or a variable speed motor control so that the respiratory rate can be similarly varied. The rate and shape of the rotating potentiometer motion can be varied by adjustments of lever length and height. The mechanism is simple and reliable.

The abolition of spontaneous respiratory activity was accomplished in one of three ways:

1) The atlanto-occipital membrane was opened and a no. 18-needle inserted through the tela choroidea into the fourth ventricle. A segment of fine polyethylene tubing was fed through the needle while the latter was withdrawn leaving the tubing in place. Injections of 1 to 2 cc. of 1 per cent procaine hydrochloride caused complete cessation of spontaneous respiratory effort in from one to two minutes, an effect which lasted for variable periods of time after which additional injections could be conveniently made through the previously placed tubing. The method will be described in detail in a separate communication (8).

2) Electrophrenic respiration, set at a rate and minute volume similar to but slightly in excess of the animal's own rate and minute volume, caused the animal to cease spontaneous respiratory activity. This sometimes occurred after only the first or second electrically induced diaphragmatic contraction. The explanation of this phenomenon is not entirely clear, but it could be produced in all but a few instances. Simple observation of the animal as well as pneumograms taken during the transition period made clear the absence of spontaneous respiratory effort when the animal was put on electrophrenic respiration (15). The mechanism of this central respiratory inhibition will be reported separately (16).

3) Large doses of nembutal intravenously were used in one experiment (no. 18) for respiratory depression in order to simulate barbiturate poisoning. Nembutal was also used in one prolonged experiment but is not well suited to that purpose.

Pneumotachograms (air flow velocity patterns) were taken with the instrument devised by Silverman (9) and Silverman and Whittenberger (10).

The experiments were designed to yield data on four points. 1) To ascertain whether normal minute volumes and blood gas tensions could be achieved with submaximal stimulation of one phrenic nerve. 2) To test the reserve of the method by measuring minute volumes and blood gas tensions during maximal stimulation of one and both phrenic nerves. 3) To clarify the relationship between peak voltage applied to the phrenic nerve and the corresponding minute volumes. 4) To ascertain whether the method could maintain respiration during prolonged acute experiments.

## RESULTS

1. *Respiratory Minute Volumes and Blood Gas Partial Pressures Under Spontaneous and Electrophrenic Respiration.* The values of respiratory minute volume and arterial oxygen and carbon dioxide tensions during spontaneous breathing and during stimulation of one or both phrenic nerves are compared in table 1. It is apparent from these data that adequate ventilation can be readily accomplished in the absence of spontaneous respiration by submaximal stimulation of one phrenic nerve.

2. *Maximal Ventilation with One or Both Phrenic Nerves.* Experiment 13 in table 1 compares the spontaneous minute volumes and arterial tensions of oxygen

and carbon dioxide with those values obtained during maximal stimulation of one phrenic nerve, after spontaneous respiration had been abolished. *Experiments 16 and 18* demonstrate the several-fold increase in ventilation that can be accomplished with bilateral stimulation and the resulting rise in oxygen pressure and fall in carbon dioxide pressure in the arterial blood.

3. *Effect of Varying Applied Peak Voltage on the Depth of Inspiration and Minute Volume.* Figure 2 demonstrates the relationship between the intensity of peak voltage and the corresponding minute volume that resulted from the stimulation of a

TABLE 1. COMPARISON OF MINUTE VOLUMES AND PARTIAL PRESSURES OF OXYGEN AND CARBON DIOXIDE OF ARTERIAL BLOOD DURING SPONTANEOUS RESPIRATION AND ELECTRICALLY INDUCED ARTIFICIAL RESPIRATION IN THE ABSENCE OF SPONTANEOUS RESPIRATION

EXP. NO. AND ANIMAL	WT.	ANESTHESIA	SPONTANEOUS RESP.			MEANS OF INHIBITING SPONTANEOUS RESP.	ELECTROPHRENIC RESPIRATION			
			M.V.	pO <sub>2</sub>	pCO <sub>2</sub>		After onset of EPR	M.V.	pO <sub>2</sub>	pCO <sub>2</sub>
	kg.		cc.	mm.	mm.		hrs.	cc.	mm.	mm.
11 Cat	3.0	Chloralose				Procaine into 4th ven- tricle	1.3 3.2 7.4 21.0	317 254 345 295	118 114 104 115 <sup>2</sup>	49 50 48 44 <sup>3</sup>
12 Cat	4.3	Chloralose	500 450	101 104	47 45	Procaine into 4th ven- tricle	1.0	530	101	45
13 Dog	12.5	Chloralose	1760	86	47	'Take over' with EPR	1.0 1.8 2.6	3940 <sup>1</sup> 3170 <sup>1</sup> 2260 <sup>1</sup>	108 96 89	30 31 45
16 Dog	13.0	Chloralose	2690	83	33	Procaine into 4th ven- tricle	0.3 1.0 1.1 2.3 <sup>2</sup>	3980 3560 3830 7160 <sup>2</sup>	87 89 86 100 <sup>2</sup>	30 31 31 24 <sup>2</sup>
18 Dog		Morphine & ure- thane	2340	34	72	'Barbiturate' poisoning	0.7 1.6 <sup>2</sup>	3340 7040 <sup>2</sup>	74 107 <sup>2</sup>	51 20 <sup>2</sup>
17 Mon- key	2.9	Nembutal	380	102	37	'Take over' with EPR	0.3 0.5	550 420	120 111	36 43

<sup>1</sup> Minute volume regulated by adjusting peak voltage.    <sup>2</sup> Both phrenic nerves stimulated.

<sup>3</sup> Single determination.

single phrenic nerve in a cat. Depth of respiration (voltage) alone was varied. The respiratory rate was kept constant throughout. *Experiment 13* in table 1 shows that the arterial oxygen and CO<sub>2</sub> tensions can likewise be regulated by the appropriate adjustment of peak voltage and thus of the minute volume.

*Pneumotachograms (tracheal air flow recordings).* Figure 3, *A* through *H*, demonstrates the effect of increasing the intensity of peak voltage upon the air flow pattern in a cat. *A* is the animal's spontaneous respiratory pattern. *B* is the air flow pattern with a peak voltage of 1.84 volts. *C*, *D*, *E*, *F*, *G* and *H* show the effect of increasing peak voltage. The three factors worthy of note which occur when peak voltage is increased are 1) the increase in the depth of inspiration and air exchange,

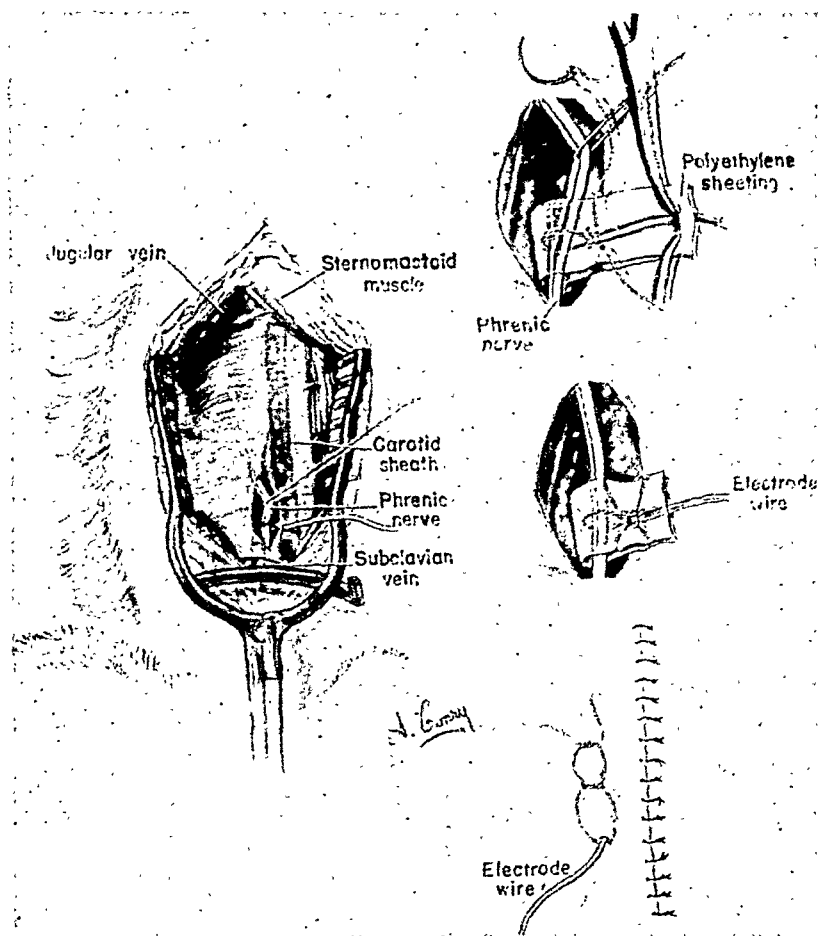


Fig. 1. TECHNIQUE OF ELECTRODE APPLICATION. A, position of bared silver wire loop surrounding phrenic trunk in dog. A silk suture firmly secures end of wire to other limb of loop. B, thin polyethylene sheeting is then placed under the nerve and folded back to enclose electrodes. A silk suture is introduced in such a way as to secure insulating sheeting firmly to electrode and to itself. C, phrenic nerve cannot escape contact with silver-wire electrode but is not compressed by it; electrode cannot stimulate any structure other than the phrenic nerve. D, end result. Lead wire emerging from puncture wound; incision is closed.

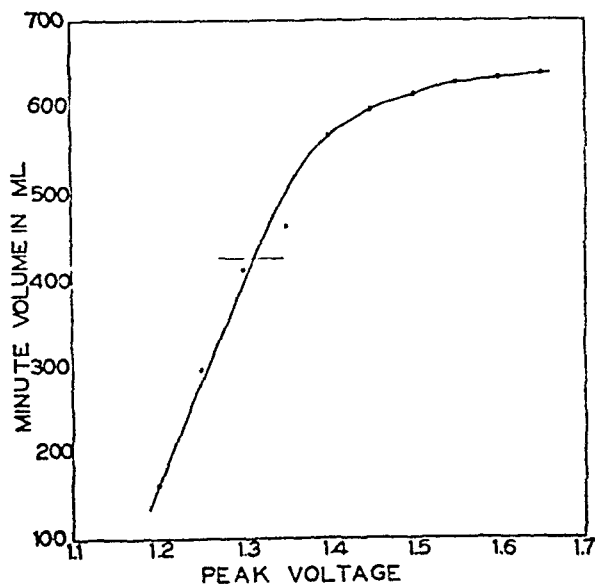


Fig. 2. RIGHT PHRENIC NERVE STIMULATION in cat in absence of spontaneous respiration (intraventricular procaine). Relationship between peak voltage applied to nerve and the corresponding minute volume is demonstrated. Crossing horizontal line indicates average minute volume value for spontaneous respiration in same animal.

2) the increase in the ratio of inspiration to the total respiratory cycle and 3) the increase in the sharpness of inspiratory effort at the higher voltages.

*Pneumotachogram of electrophrenic respiration by 'remote' control.* Figure 3 *I* is a tracing from the same animal during electrophrenic respiration by the 'remote' control technique (11, 12) after spontaneous breathing had been abolished by the injection of procaine into the fourth ventricle. The secondary coil was not actually

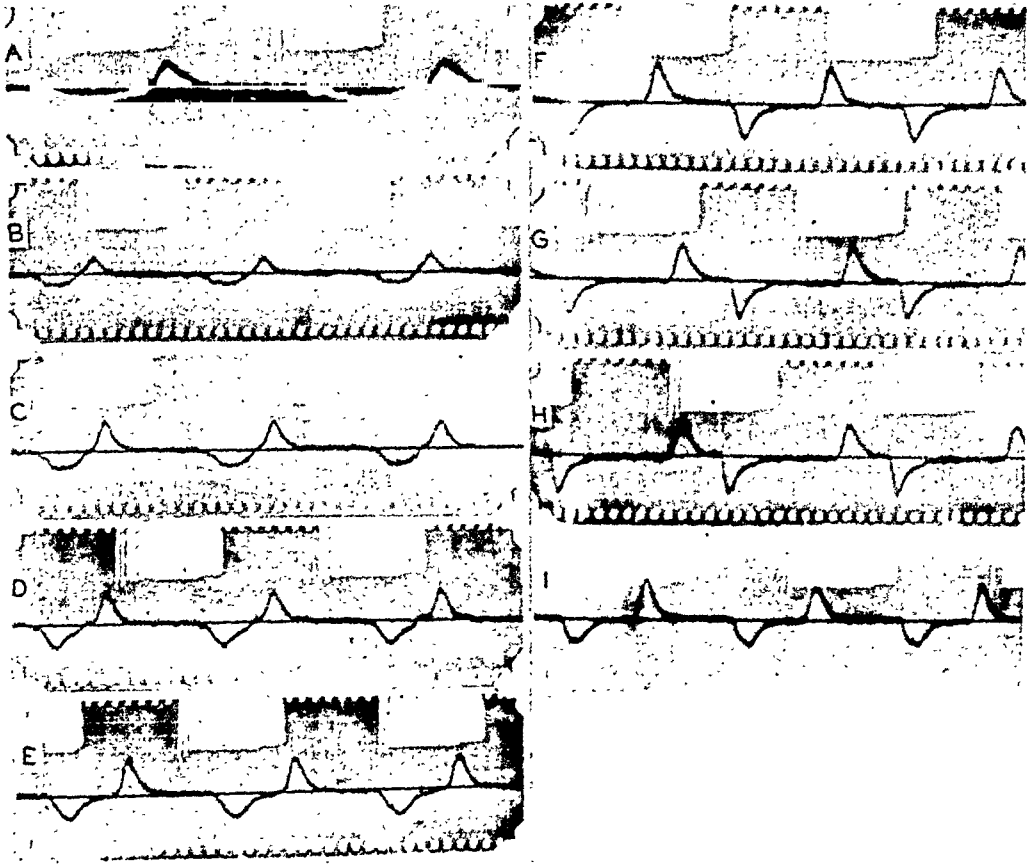


Fig. 3. EFFECTS ON AIR FLOW RECORDINGS of increasing peak voltage applied to right phrenic nerve of cat. A, spontaneous respiration. B, phrenic stimulation with peak voltage of 1.84. C, peak voltage of 1.94 volts. D, peak voltage of 2.04. E, peak voltage of 2.16. F, peak voltage of 2.50 volts. G, peak voltage of 4.00. H, peak voltage of 10.00. I, phrenic stimulation by "remote control" technique. Area below base line indicates inspiration; area above the base line indicates expiration. Area below base line is directly proportional to amount of inspired air.

implanted under the skin but was separated from the primary by a distance of more than one inch.

4. *Prolonged Experiments.* Three prolonged experiments were carried out, using 2 cats and 1 dog. In the first, a cat, spontaneous breathing was abolished by large intravenous doses of sodium nembutal. It was possible to maintain the animal for 12 hours on electrophrenic respiration. Due to the fact that large doses of barbiturate were necessary, the circulation was profoundly depressed and it was felt that this was the cause of the cat's death rather than failure of the neuromuscular mechanism of the diaphragm. The second experiment lasted 16 hours and was probably

terminated by a pulmonary embolus which arose from the dog's femoral vein at the site of multiple venepunctures and an indwelling needle. The third prolonged experiment was performed in a cat and lasted 22 hours. The animal was maintained in excellent condition during this entire period by the electrical stimulation of only the right phrenic nerve while respiration was abolished by the intermittent injection of 1.0 per cent procaine into the fourth ventricle. At the end of 21 hours the partial pressure of  $O_2$  in the blood was 115 mm. Hg and that of  $CO_2$  was 44.5 (single determination, *experiment 11* in table 1). The animal was supported by Ringer's solution given intravenously at intervals throughout the experiment. The experiment was unfortunately terminated by tracheal obstruction in the form of an unsuspected mucous plug in the tracheal cannula. The diaphragm was contracting vigorously at that time.

#### DISCUSSION

It is apparent from the data in table 1 that adequate aeration, as reflected by minute volume, arterial blood oxygenation and the elimination of carbon dioxide, can be achieved by the submaximal electrical stimulation of a single phrenic nerve in the absence of spontaneous respiratory activity. The data in table 1 and figures 2 and 3 demonstrate that minute volumes, appreciably in excess of those which the animal spontaneously performs to maintain adequate aeration under anesthesia, can be achieved by the use of submaximal stimulation of one phrenic nerve. Maximal stimulation of one phrenic nerve increases ventilation and bilateral phrenic stimulation produces still greater minute volumes.

Table 1, figures 2 and 3 indicate that the tidal volume and minute volume can be regulated satisfactorily by adjusting the voltage applied to the phrenic nerve in such a way as to cause the diaphragm to contract feebly or forcefully as may be desired. In addition, the ratio of the length of inspiration to the length of the total cycle can be varied from 25 to 72 per cent (fig. 3). The sharpness of inspiratory effort can likewise be regulated. Thus a variety of respiratory rates and air flow contours are obtainable.

The prolonged experiments indicate that the neuromuscular mechanism is capable of sustaining prolonged electrical stimulation and the method is thus capable of maintaining life for prolonged periods of time. It is our opinion that in these experiments the failure at the end of the prolonged periods was not due to failure of the neuromuscular mechanism. Under conditions not necessitating deep barbiturate narcosis or central nervous system interference, it is anticipated that stimulation could be successfully maintained for considerably longer periods. The work of Fender (13) is of considerable interest in this connection. He implanted silver electrodes on both splanchnic nerves of the dog and effectively stimulated them with from 6 to 8 volts, 8 hours a day, 6 days a week, for  $5\frac{1}{2}$  months. At the end of that time the nerves responded well to stimulation and showed no anatomic evidence of injury.

Experimentally, the method may prove useful in several ways: *a*) production of regular breathing in hemodynamic studies, when irregular breathing often clouds the interpretation of vascular data, *b*) elucidation of neurogenic control of



breathing, as exemplified by marked differences between this method and the usual positive pressure respiration (to be reported separately), *c*) separation of ventilation into diaphragmatic and intercostal components, *d*) separation of respiratory weakness into central and neuromuscular components, and *e*) ease of obtaining reproducible ventilation patterns in studies of lung absorption of inhaled gases, aerosols etc.

Other observations to be reported elsewhere (14) have demonstrated that, as was anticipated, the human phrenic nerve and diaphragm act in much the same fashion as do those of the experimental animal. The phrenic nerve in man lies directly behind the sternocleidomastoid muscle on the belly of the anterior scalene. Exposure of the nerve can be accomplished in a matter of minutes under local anesthesia. It would be feasible, therefore, to apply this method of artificial respiration to human patients if, eventually, the advantages of the method are established in man.

Although much preliminary work remains to be done, the potential advantages of this method have been considered. These include *a*) portability and low cost of the apparatus, *b*) ease of nursing care and freedom of movement of the patient, and *c*) therapeutic effect of keeping a partially paralyzed diaphragm active. Candidates for this method would be those with intact phrenic nerves, for example patients with bulbar depression or paralysis or high spinal anesthesia. Possible use of the method in patients with anterior horn cell disease must await further study.

Prerequisites to extensive use of the electrophrenic method of artificial respiration in man are *a*) clear evidence that electrical stimulation of the phrenic does not injure the nerve or its anterior horn cells and *b*) development of a means of stimulating the phrenic without an operative procedure.

Chaffee and Light (11, 12) have devised a method for the 'remote' stimulation of the nervous system. The method was also used by Fender (13). 'Remote' stimulation is accomplished by imbedding a small secondary coil under the skin with one indifferent electrode and one fine stimulating electrode leading to the nerve that it is desired to stimulate. Later, it is possible to stimulate the nerve in question by bringing the primary coil near the implanted secondary (but external to the animal or patient) and thereby induce a known current in the previously implanted secondary coil. In this way, artificial respiration could be induced without the necessity for continued direct contact between the source of current and the phrenic nerve. This obviates the necessity for having a lead wire emanate from the wound. In *experiment 12* a cat was stimulated by such a 'remote control' technique and figure 3 *I* is the pneumotachographic tracing taken while this type of artificial respiration was being produced. The disadvantage of this technique is the fact that the implanted coil must be of considerable size to insure an adequate stimulus and this disadvantage probably outweighs the gain from avoiding the presence of a single lead wire. For experimental application the most satisfactory method at present is the technique of implanting a single electrode.

#### SUMMARY

It is feasible to produce artificial respiration by the electrical stimulation of one or both phrenic nerves. By this means, the animal's spontaneous minute volume

can easily be exceeded and the tensions of oxygen and carbon dioxide in arterial blood maintained at satisfactory levels for as long as 22 hours in the absence of spontaneous respiratory activity. The rate and depth of respiration and the contour of respiratory air flow can be readily modified within broad limits.

Clinical and experimental implications of the method have been presented.

The authors wish to express their thanks to Dr. Robert Schwab and Dr. Jason Mixter for their encouragement in the early phases of this work and to Mrs. Harriet A. Kriete for technical assistance.

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# BLOOD SUGAR RESPONSE TO ANOXIA DURING ACCLIMATIZATION

EDWARD J. VAN LIERE, J. CLIFFORD STICKNEY AND DAVID W. NORTHUP

*From the Department of Physiology, West Virginia University, School of Medicine*

MORGANTOWN, WEST VIRGINIA

**T**HERE are several objective physiologic criteria which can be used to determine adaptation to altitude. The most important of these are: an increase in the total ventilation of the lungs; changes in alveolar  $\text{CO}_2$  and  $\text{O}_2$  tensions; alterations in blood chemistry; an increase in the amount of hemoglobin and number of red blood cells; certain changes in the circulatory system and in gastro-intestinal function.

Acclimatization is an involved process and is not at all well understood. It is desirable that as many criteria as possible be established. On this account it seemed worthwhile to study the effect of acclimatization on blood sugar response to anoxic anoxia.

## METHODS

Six dogs were used in these experiments. Throughout the course of the study they were fed an adequate diet including table scraps, purina dog chow and a liberal amount of milk. Before being subjected to test for blood sugar response they were fasted for 24 hours. The ranges of control-blood sugar values in the 6 dogs before exposure to anoxia are shown in table 1.

The animals were subjected to a barometric pressure of 254 mm. of Hg (approximate altitude of 28,000 ft.) in a low-pressure chamber for 15 minutes and blood sugar determinations were then made (Folin-Wu method.) Several such determinations were made at weekly intervals. After the control figure had been established, the animals were exposed to intermittent anoxia of 303 mm. Hg (approximate altitude of 24,000 ft.) 4 hours each day, including Sunday, until they became acclimatized as shown by the fact that exposure to a simulated altitude of 28,000 feet did not cause elevation of the blood sugar.

Daily exposures were then discontinued so that the animals might lose their acclimatization. In order to follow the process of deacclimatization, they were tested by a single exposure to a barometric pressure of 254 mm. Hg for a 15-minute period at varying intervals, but not, as a rule, oftener than once a week.

## RESULTS

Five of the 6 animals showed acclimatization as evidenced by the fact that when subjected to a pressure of 254 mm. Hg for 15 minutes, no significant elevation of blood sugar occurred. One animal which was extremely sensitive to anoxia showed only partial acclimatization.

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Received for publication August 18, 1948.

The results of 3 representative animals are shown in the accompanying figures. *Dog 2* (fig. 1) lost most of its acclimatization (as regards response of blood sugar to anoxia) within a period of about 15 days. On the other hand *dog 4* (fig. 2) started losing its acclimatization after about 6 weeks, but lost it slowly and did not deacclimatize until about 5 months had elapsed. *Dog 5* (fig. 3) still showed acclimatization 7 months after it had been exposed to intermittent anoxia; further determinations were not made on this animal. The data obtained from the 2 remaining dogs were

TABLE I. RANGE OF CONTROL BLOOD SUGARS ON DOGS BEFORE EXPOSURE TO ANOXIA

DOG NO.	MG/PER 100 ML.	NO. OF DETERMINATIONS
2	91-110	9
3	87-111	9
4	108-128	9
5	105-124	9
6	107-122	10
8	101-105	2

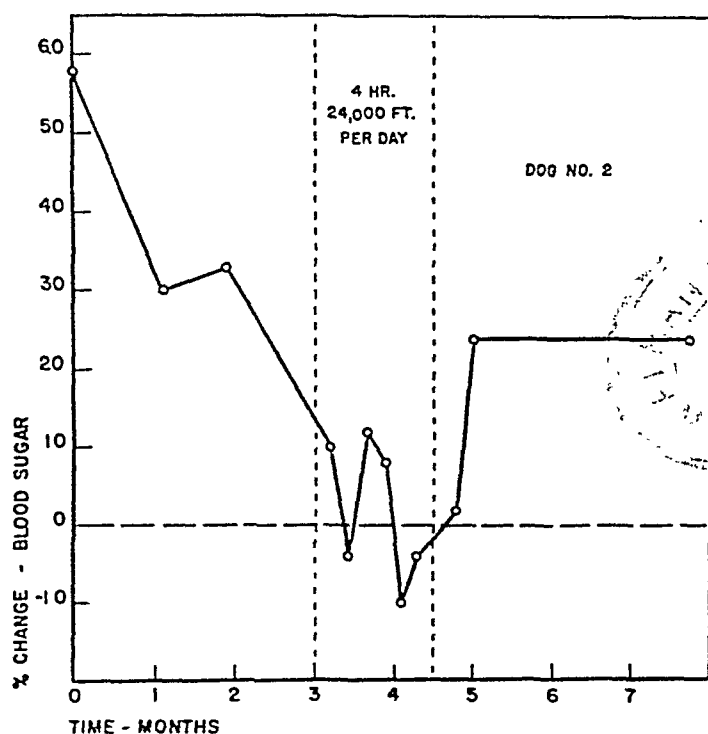


Fig. 1. ACCLIMATIZATION of the hyperglycemic response to 28,000-ft.

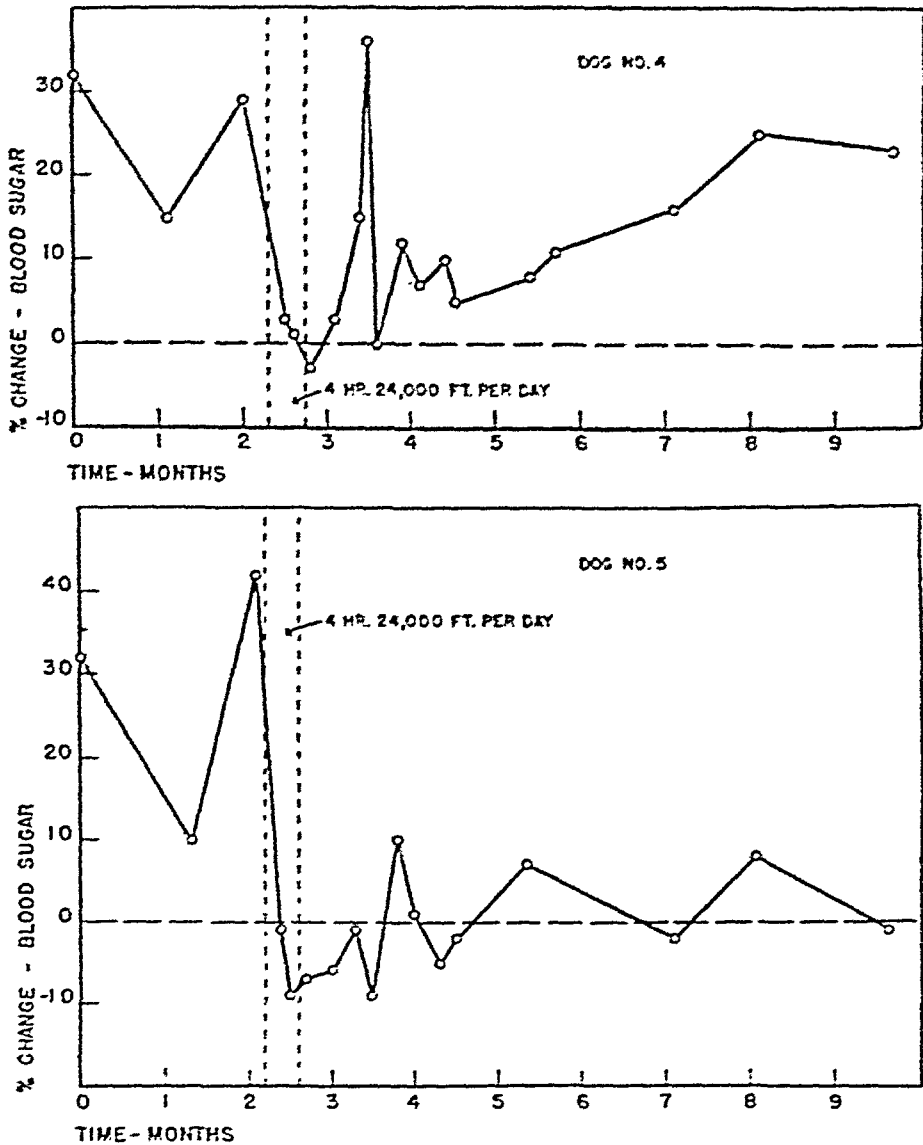
as follows: the results in one of the dogs were indeterminate at the end of 2 months; the other animal had hardly lost any of his acclimatization at the end of 2 months.

#### DISCUSSION

It is of interest that it took only a relatively short period of time (about a week) to acclimatize these animals, as far as the blood sugar response to anoxia was concerned. In work reported from this laboratory (1) on the effect of intermittent anoxia on the hemoglobin and on the number of red blood cells, it was found that there was no significant rise in either until nearly three weeks had elapsed. This

same time element was found also in experiments we reported concerning acclimatization and gastric emptying (2). These experiments were also performed on dogs.

In order to demonstrate that there is virtually an acclimatization for every altitude and to show that the adrenal glands were not exhausted, the acclimatized animals were subjected to a pressure of 208 mm. Hg (approximate altitude of 32,000



Figs. 2 (upper) and 3 (lower). ACCLIMATIZATION of the hyperglycemic response to 28,000 ft.

ft. for 15 minutes). Five of the 6 dogs showed a significant rise in blood sugar. However, the animal which showed only partial acclimatization died within a few minutes after being exposed to this simulated extreme altitude.

It is noteworthy that 2 of the surviving animals did not deacclimatize—at least during the time they were under observation. It is difficult to account for this

phenomenon. There are several factors which might be mentioned: *a*) anoxia disturbed the balance between the vago-insulin and the sympathico-adrenal systems; *b*) anoxia impaired the function of the liver depots so that the sugar was not released; *c*) anoxia changed the threshold of the response of blood sugar to altitude. All of these factors are more or less speculative and, as far as we are aware, no definite proof for any of them exists.

If the vago-insulin and the sympathico-adrenal balance were disturbed by the anoxia, it is difficult to see why these mechanisms apparently were still in balance when the animals were subjected to more severe degrees of anoxia than those to which they had been acclimatized. Relative to the effect of acclimatization on the liver, Gellhorn and Packer (3) working with unanesthetized rabbits reported that brief periods of anoxia tend to antagonize the effect of insulin and cause a quicker recovery of the blood sugar to normal values. Prolonged anoxia, however, leads to an aggravation of the hypoglycemia and a progressive fall of the blood sugar curve. These authors feel that the failure of prolonged anoxia to bring about a recovery of the blood sugar curve is due to the inability of epinephrine to liberate glucose from the liver. The question arises whether this could be applied to acclimatized animals. Lastly, anoxia may change the threshold of the response of blood sugar to altitude by some mechanism not as yet understood. It would not necessarily have to be central in character.

It is hard to believe that those animals which did not deacclimatize were permanently acclimatized as far as the blood sugar response to anoxia was concerned. It is known that animals upon return to sea-level lose their characteristic physiologic changes associated with high altitude adaptation. For example, when acclimatized animals are brought down to sea-level, the number of red blood cells and the amount of hemoglobin return to normal values within a relatively short time. It is quite likely that some organs or body systems retain their acclimatization longer than do others, but it does not seem likely that they would retain it indefinitely.

It should be mentioned that subjectively the animals looked more at ease at altitude after they had been acclimatized than they did at the beginning of the experiment. Their muscular coordination, too, upon removal from the low-pressure chamber was much better. The animals did not lose any appreciable amount of weight in the course of the experiments and, save the one which died, remained healthy and vigorous throughout. There was no increase in the amount of hemoglobin in the 4 dogs which showed acclimatization (in response of blood sugar to anoxia) within a period of about a week. Two animals, however, which were subjected to intermittent anoxia for a longer period showed, as would be expected, an appreciable rise in the amount of hemoglobin.

#### SUMMARY

After the normal response of blood sugar to anoxia (254 mm. Hg) was determined in 6 dogs, the animals were subjected to a pressure of 303 mm. Hg for 4 hours a day. Five of the 6 animals became acclimatized as far as the blood sugar response was concerned as evidenced by the fact that there was no increase when exposed to

a pressure of 254 mm. Hg. Several animals deacclimatized within a relatively short time, but one animal showed considerable acclimatization at the end of 2 months, and another animal apparently was still acclimatized at the end of a 7-month period.

The technical assistance of W. V. Crabtree during part of this work is gratefully acknowledged.

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# EFFECT OF MUSCLE WORK UPON TOLERANCE OF EVISCERATED RAT FOR GLUCOSE

DWIGHT J. INGLE AND JAMES E. NEZAMIS

*From the Research Laboratories, The Upjohn Company*

KALAMAZOO, MICHIGAN

IT HAS been shown (1) that muscular work accelerates the rate of fall of blood glucose in eviscerated and eviscerated-nephrectomized rats. The present experiments demonstrate the marked effect of muscle work upon the glucose load which the eviscerated rat can tolerate in the presence and absence of insulin.

## METHODS

Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At a weight of 185 to 205 grams, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. Asepsis was preserved in this operation. When the animals reached a weight of 250 ( $\pm 2$ ) grams they were anesthetized (intraperitoneal injection of 18 mg. of cyclopentenyl-allyl-barbituric acid sodium) and eviscerated by the procedure of Ingle and Griffith (2). Hemostasis was attained by applying a gelatin sponge (Gelfoam, Upjohn) saturated with a solution of thrombin to the stumps of the esophagus, colon, ligated vessels and between the muscle and skin when the incisions were closed.

Immediately following the operation, the animals were prepared for the stimulation of muscle according to Ingle (3) and with the following modifications. A Nerve Stimulator (Model B, Upjohn) was used to stimulate muscle at the rate of 5 times per second. The duration of each pulse was 20 msec. and the intensity was 20 ma. In *experiment 1*, the stimulus was applied to the left leg only; in *experiment 2*, the stimulus passed from the right back foot to the contralateral back foot thereby activating the entire musculature of both legs. The work of the left gastrocnemius was registered on automatic work recorders. Each recorder revolution represented approximately 400-gram centimeters of work. The animals were enclosed in a cabinet with temperature constant at 26.5° ( $\pm .5$ ) C.

Solutions of glucose (C.P. Dextrose, Merck) with and without insulin (crystalline zinc, Lilly) were infused into the jugular vein at a constant rate by means of a continuous injection machine which delivered fluid from each of 12 syringes at the rate of 20 cc. in 24 hours. The glucose load is expressed as milligrams of glucose per 100 grams of rat per hour (mg/100/h.). Insulin was given at the rate of 4 U per 24 hours. The infusions covered a period of 2 hours.

Analyses of glucose were made on tail blood by the method of Miller and Van Slyke (4).

## EXPERIMENTS AND RESULTS

*Experiment 1* (fig. 1) was a study of the effect of stimulating the gastrocnemius muscle of one leg upon the tolerance of the eviscerated rat for glucose. The following



glucose loads were covered: 0, 16, 24, 36, 44, 48, 52, 70, 100, 110, 120 and 130/100/h. The following experimental conditions with 8 rats per group were represented: no work, no insulin; work, no insulin; no work, insulin; and work, insulin. During the 2-hour period the glucose load tolerated by the non-working rat without insulin was approximately 16/100/h.; with insulin the value was increased to 72/100/h. Under similar conditions the stimulation of one leg in rats without insulin increased the tolerance for glucose to approximately 48/100/h.; with insulin the tolerance was raised to approximately 110/100/h.

*Experiment 2* (fig. 2) was a study of the effect of activating all of the musculature of both back legs upon the tolerance of the eviscerated rat for glucose. The following glucose loads were covered: 50, 72, 100, 120, 140, 160, 180, 200, 220, 240, 260 and 280/100 h. Working rats were studied with and without insulin. The average

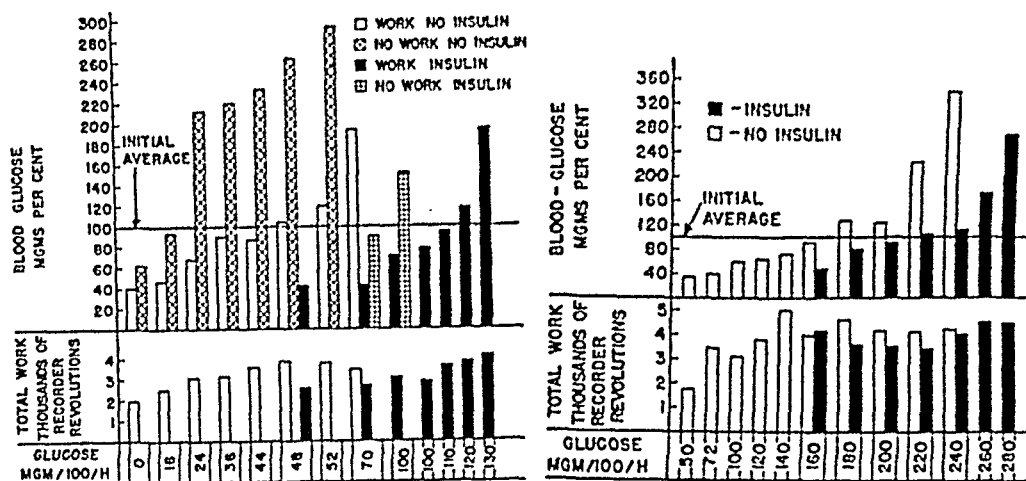


Fig. 1 (left). EFFECT OF STIMULATING THE GASTROCNEMIUS MUSCLE of one leg upon the glucose tolerance of eviscerated rats with and without insulin. Average level of blood glucose at the end of 2 hours. Eight rats per group.

Fig. 2 (right). GLUCOSE TOLERANCE OF EVISCERATED RATS subjected to stimulation of both back legs with and without insulin. Average level of blood glucose at the end of 2 hours. Eight rats per group.

glucose tolerance for rats without insulin was approximately 160/100/h.; with insulin this value was increased to approximately 220/100/h.

As shown in figures 1 and 2 the amounts of work recorded during 2 hours of stimulation were roughly proportional to the glucose load in those animals which did not receive insulin. When the level of blood glucose was suppressed by the administration of insulin the work performance was definitely depressed. There was no tendency for the addition of insulin to improve work performance under these experimental conditions.

#### DISCUSSION

Under resting conditions the tolerance of the eviscerated rat for glucose is very low, although it can be increased somewhat by insulin. It is probable that this low requirement for glucose is due in major part to inactivity. During muscular work

the requirement for glucose can be increased ten-fold without the addition of insulin. We have not determined the fate of the glucose or the changes in carbohydrate stores in the peripheral tissues. It seems reasonable to draw the tentative conclusion that the stimulation of muscle causes a marked increase in the utilization of glucose without any change in the insulin content of the body fluids. It is also reasonable to suggest that these rats were insulin-deficient following the removal of the pancreas by evisceration.

The addition of insulin increased the tolerance for glucose by approximately the same load (60/100/h.) in the resting animal, in the animal subjected to stimulation of the gastrocnemius of one leg and in animals subjected to stimulation of both hind legs. This may have been fortuitous. The administration of insulin did not improve work performance. On the contrary, when the level of blood glucose was lowered by insulin the performance of work was somewhat depressed. It is possible that the action of insulin favored pathways of glucose conversion which competed with the contracting muscle for the glucose.

The effect of work upon the carbohydrate requirement of the eviscerated rat should be explored further in animals made severely diabetic prior to evisceration. A full interpretation of the results would require tracing the pathways of carbohydrate utilization during rest and work.

#### SUMMARY

The effect of work upon the tolerance of the eviscerated rat for intravenously administered glucose was studied during a period of 2 hours. In *experiment 1*, the gastrocnemius muscle of one leg was stimulated to lift 100 grams at the rate of five times per second. The average glucose load tolerated by the non-working rat without insulin was approximately 16/100/h.; with insulin the value was 72/100/h. Under similar conditions the stimulation of one leg in rats without insulin increased the tolerance for glucose to approximately 48/100/h.; with insulin the approximate value was 110/100/h.

In *experiment 2*, the stimulus passed from one back foot to the contralateral back foot thereby activating the entire musculature of both hind legs. The average value for glucose tolerance without insulin was approximately 160/100/h.; with insulin the approximate value was 220/100/h.

Work performance was roughly proportional to the glucose load in those animals which did not receive insulin. The administration of insulin suppressed work performance at the lower glucose loads. In no instance did the administration of insulin enhance the ability of the eviscerated rat to work.

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# EFFECTS OF THE PITUITARY GROWTH AND ADRENOCORTICOTROPIC HORMONES ON THE URINARY GLUCOSE, NITROGEN AND KETONE BODIES OF DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET<sup>1</sup>

LESLIE L. BENNETT AND BARBARA LAUNDRIE

*From the Institute of Experimental Biology and the Division of Physiology,  
University of California*

BERKELEY, CALIFORNIA

PREVIOUS work from this laboratory has demonstrated that the administration of pure adrenocorticotrophic hormone enhanced both the glycosuria and urinary nitrogen excretion of diabetic rats which were maintained on a stock diet (1). The hypophyseal growth hormone produced nitrogen retention but had little effect upon the glycosuria of such animals. Either adrenocorticotrophic hormone or growth hormone increased the ketonemia and ketonuria of fasted normal rats (2). Adrenocorticotrophic hormone increased both the glycosuria and urinary nitrogen excretion of diabetic rats which were maintained on a carbohydrate-free diet (3). The effect of growth hormone under these conditions has not been investigated previously. It is the purpose of this paper to present some experiments in which these two hypophyseal hormones were administered to diabetic rats, which were maintained on a carbohydrate-free diet, and the resulting changes in the urinary excretion of glucose, ketone bodies and nitrogen were investigated. The experiments with adrenocorticotrophic hormone are in addition to those previously reported (3).

## METHODS

All animals used were male rats of the Long-Evans strain between 55 to 65 days of age at the time of production of diabetes. Diabetes was produced by the intraperitoneal injection of 200 mg/kg. body weight of alloxan monohydrate (Eastman) on each of two successive days. Ten rats which had a persistent and uniform glycosuria while on the carbohydrate-free diet<sup>2</sup> were selected for study. Although the degree of glycosuria was uniform from day to day in each animal, it dif-

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Received for publication July 19, 1948.

<sup>1</sup> Aided by grants from the American Cancer Society (through the National Research Council, Committee on Growth), the National Institute of Health, RG #409, and The Research Board, University of California. The following materials were generously contributed: Crystalline B vitamins and  $\alpha$ -tocopherol from Merck and Company, Inc., Rahway, N. J.; synthetic pteroylglutamic acid (Liver *L. casei* factor) and liver syrup (Lab. No. 7-6737) from Lederle Laboratories, Inc., Pearl River, N. Y.; synthetic *d*-biotin from Hoffman La-Roche, Inc., Nutley, N. J.

<sup>2</sup> The carbohydrate-free diet consisted of alcohol-extracted casein 71%, hydrogenated vegetable oil (Crisco) 24%, salts No. 4 (6) 4% and liver syrup and B vitamin mixture one %. This furnished the following amounts of vitamins/kg. diet: 2-methyl-1,4-naphthoquinone 5 mg., thiamine HCl 5 mg., riboflavin 10 mg., pyridoxine HCl 5 mg., *p*-aminobenzoic acid 10 mg., nicotinic acid 20 mg., calcium pantothenate 50 mg., inositol 400 mg., pteroylglutamic acid 5.5 mg., synthetic *d*-biotin 0.3 mg. and choline chloride one gm. One cc. of a fat-soluble vitamin mixture was fed at about the mid-point of the metabolic study period. This consisted of 6 mg.  $\alpha$ -tocopherol (Merck), 115 chick units vitamin D and 800 USP units vitamin A in 650 mg. corn oil (Mazola).

ferred from one animal to another, varying from 220 mg. to 3980 mg./day. Thus, the effect of the hormones could be evaluated in animals with varying degrees of severity of diabetes.

By trial and error it was determined how much food would be consumed completely by each animal each day and this amount was fed daily throughout the experiment. Therefore, with but one exception, the food intake was constant for each animal throughout the entire period of observation. One rat during the injection of adrenocorticotrophic hormone failed to eat as much as it had during the pre-injection control period. During the post-injection control period it was fed daily an amount of food equal to the average daily consumption during the injection period.

The animals were kept in individual metabolism cages and were fed at the same time each morning. Urines were collected through large ribbed glass funnels into wide-mouthed Erlenmeyer flasks which contained a layer of toluene as a preservative. Feces were separated from the urine by perforated porcelain discs. Collections were made each morning by washing down the funnels with 300 to 400 cc. of distilled water.

Glucose was determined by the Somogyi (4) method and is reported as total reducing substances. Correction for non-fermentable reducing substances were not carried out, since studies with yeast fermentation have shown that the urinary content of non-fermentable reducing substances is as negligible in animals upon this diet as it is in animals upon the stock diet (1). Urinary nitrogen was determined by the micro-Kjeldahl procedure. In these experiments fecal nitrogen analyses were not done. Previous unpublished observations have confirmed that the fecal nitrogen excretion is constant as long as the dietary intake is constant and, in addition, have shown that growth hormone or adrenocorticotrophic hormone administration is without effect upon the fecal nitrogen excretion. Thus, changes in urinary nitrogen excretion may be taken as a valid index of changes in nitrogen balance under the condition employed in these experiments. Urinary ketone bodies were determined by the method of Van Slyke (5) with Denigé's reagent. In two of the experiments with growth hormone ketone bodies were not determined (*Exp. 5 and 6*).

The growth hormone and adrenocorticotrophic hormone<sup>3</sup> used were prepared according to the previously published method (7, 8) and were administered intraperitoneally at a dose level of 3 mg./day, three injections of one mg. each being given at intervals of about five hours during the day.

Before administering a hormone, the urinary excretion of glucose, nitrogen and ketone bodies was determined for a pre-injection control period of from 8 to 18 days. The duration of the period of hormone administration in most instances was five days. In some cases, after the administration of one hormone for a five-day period, injection of the other hormone was started immediately without an intervening control period. In all cases following the cessation of injection a post-injection control period of from five to nine days was carried out, the animals being followed for the longer period when the degree of glycosuria and urinary nitrogen excretion did not immediately return to the pre-injection level.

#### RESULTS AND COMMENT

The data of the experiments are presented in tables 1, 2 and 3 and in figures 1 and 2. Figure 2 contains data in addition to that incorporated in the tables and figure 1 shows the type of day-by-day variations that were encountered. In the graphic presentation, individual daily values representing the response of 2 individual rats to each hormone are shown. In the tabular presentation, the average excretion during the injection period has been compared statistically with the average excretion during the control period. A *p* value of 0.05 or less was considered to be significant. Only the average of the pre-injection control period is shown in the table except when the pre- and post-injection control periods were different, in which case the mean of each is given. There is one exception. In the case of *experiment 9*, only the post-injection control data were presented. This is the animal that reduced its food intake during the injection period.

Inspection of table 1 shows that the administration of growth hormone did not

<sup>3</sup> The authors are indebted to Doctor Choh Hao Li for the preparation of the hormones.

produce a significant increase in glycosuria in any of the 6 animals. Figure 2 demonstrates that, even when the glycosuria had been enhanced by prior administration of adrenocorticotrophic hormone, growth hormone did not maintain the glycosuria at the

TABLE 1. EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF GLUCOSE BY DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

EXP. NO.	GM. DIET/DAY	HORMONE/DAY IN MG.	URINARY GLUCOSE IN MG/DAY			
			Control period	Injection period	Change	$P^4$
1	10	3 GH	220 $\pm$ 30 <sup>2</sup> (8) <sup>2</sup>	320 $\pm$ 80 (5)	+100	0.20
2	14	3 GH	480 $\pm$ 110 (8)	820 $\pm$ 150 (5)	+340	0.07
3	12	3 GH <sup>1</sup>	2040 $\pm$ 90 (9)	2280 $\pm$ 100 (5)	+240	0.10
4	12	3 GH <sup>1</sup>	2930 $\pm$ 180 (7)	3020 $\pm$ 180 (5)	+90	0.30
5	16	3 GH	3300 $\pm$ 100 (17)	3330 $\pm$ 200 (6)	+30	0.85
6	16	3 GH	3660 $\pm$ 70 (17)	3780 $\pm$ 150 (6)	+120	0.40
8	12	3 ACTH <sup>1</sup>	930 $\pm$ 70 (8)	2930 $\pm$ 150 (5)	+2000	<0.01
9	7.6	3 ACTH <sup>1</sup>	1770 $\pm$ 140 (9)	2470 $\pm$ 230 (5)	+700	0.01
10	10	3 ACTH	230 $\pm$ 40 (9)	760 $\pm$ 140 (5)	+530	<0.01
11	14	3 ACTH	560 $\pm$ 120 (9)	2040 $\pm$ 210 (5)	+1480	<0.01
12	12	3 ACTH	2040 $\pm$ 90 (9)	2010 $\pm$ 190 (5)	-30	0.90
			1390 $\pm$ 210 (4)		+620	00.04

<sup>1</sup> First day only 2 mg.    <sup>2</sup> Number of days of observation.    <sup>3</sup> Standard deviation of the mean

<sup>4</sup> From Fisher's (9) table of  $t$ .

TABLE 2. EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF NITROGEN BY DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

EXP. NO.	GM. DIET/DAY	HORMONE/DAY IN MG.	URINARY NITROGEN IN MG/DAY			
			Control period	Injection period	Change	$P^4$
1	10	3 GH	780 $\pm$ 15 <sup>3</sup> (8) <sup>1</sup>	684 $\pm$ 15 (5)	-96	<0.01
2	14	3 GH	1062 $\pm$ 15 (8)	945 $\pm$ 19 (5)	-117	<0.01
3	12	3 GH <sup>2</sup>	948 $\pm$ 21 (9)	849 $\pm$ 16 (5)	-99	<.01
4	12	3 GH <sup>2</sup>	891 $\pm$ 18 (7)	874 $\pm$ 41 (5)	-17	0.65
			1041 $\pm$ 35 (5)		-167	<0.01
5	16	3 GH	1369 $\pm$ 16 (18)	1152 $\pm$ 39 (6)	-217	<0.01
6	16	3 GH	1297 $\pm$ 17 (18)	1209 $\pm$ 29 (6)	-88	0.02
8	12	3 ACTH <sup>2</sup>	876 $\pm$ 17 (8)	1058 $\pm$ 18 (5)	+182	<0.01
9	7.6	3 ACTH <sup>2</sup>	612 $\pm$ 25 (9)	745 $\pm$ 61 (5)	+133	0.02
10	10	3 ACTH	800 $\pm$ 17 (9)	870 $\pm$ 19 (5)	+70	0.02
11	14	3 ACTH	1074 $\pm$ 19 (9)	1207 $\pm$ 22 (5)	+133	<0.01
			1183 $\pm$ 30 (6)		+24	0.50
12	12	3 ACTH	948 $\pm$ 21 (9)	1021 $\pm$ 36 (5)	+73	0.06

<sup>1</sup> Number of days observation.    <sup>2</sup> First day only 2 mg.    <sup>3</sup> Standard deviation of the mean.

<sup>4</sup> From Fisher's (9) table of  $t$ .

higher level. On the other hand, adrenocorticotrophic hormone produced a significant increase in glycosuria in 4 of the 5 animals to which it was administered. It is of interest to note that in *experiment 12*, in which there was no increase when compared to the pre-injection control period, the diabetes was of considerable severity,

the rat initially excreting more than 2 gm. of glucose per day in spite of the absence of any dietary carbohydrate. This observation is in conformity with those previously

TABLE 3. EFFECT OF GH AND OF ACTH ON THE URINARY EXCRETION OF KETONE BODIES OF DIABETIC RATS MAINTAINED ON A CARBOHYDRATE-FREE DIET

EXP. NO.	GM. DIET/DAY	HORMONE/DAY IN MG.	URINARY KETONE BODIES IN MG/DAY				
			Control period		Injection period		Change
1	10	3 GH	4.1 ± .82 <sup>2</sup>	(7) <sup>1</sup>	2.3 ± .70	(5)	-1.8
2	14	3 GH	4.7 ± 1.13	(7)	5.0 ± 1.94	(5)	+0.3
3	12	3 GH <sup>3</sup>	3.8 ± .64	(8)	15.8 ± 6.65	(5)	+12.0
4	12	3 GH <sup>3</sup>	3.9 ± .82	(7)	111.1 ± 28.6	(5)	+107.2
7	12	3 GH	3.3 ± .81	(7)	43.1 ± 23.0	(4)	+39.8
8	12	3 ACTH <sup>3</sup>	3.0 ± .66	(8)	22.7 ± 13.6	(5)	+19.7
9	7.6	3 ACTH <sup>3</sup>	5.9 ± 3.63	(8)	67.6 ± 15.7	(5)	+61.7
10	10	3 ACTH	3.7 ± .84	(8)	3.1 ± 1.10	(5)	-0.6
11	14	3 ACTH	4.6 ± 1.00	(8)	4.9 ± .83	(5)	+0.3
			2.1 ± .40	(7)			+2.8
12	12	3 ACTH	1.6 ± .68	(5)	3.1 ± 1.78	(5)	+1.5
13	14	3 ACTH <sup>3</sup>	49.5 ± 18.8	(7)	254.0 <sup>4</sup> ± 23.4	(4)	+204.5

<sup>1</sup> Number of days observation.

<sup>2</sup> Standard deviation of the mean.

<sup>3</sup> First day only 2 mg.

<sup>4</sup> Food consumption reduced to 9 gm. per day during the injection period. Died at end of 5th day of injection. Glucose and nitrogen data not presented.

<sup>5</sup> From Fisher's (9) table of *t*.

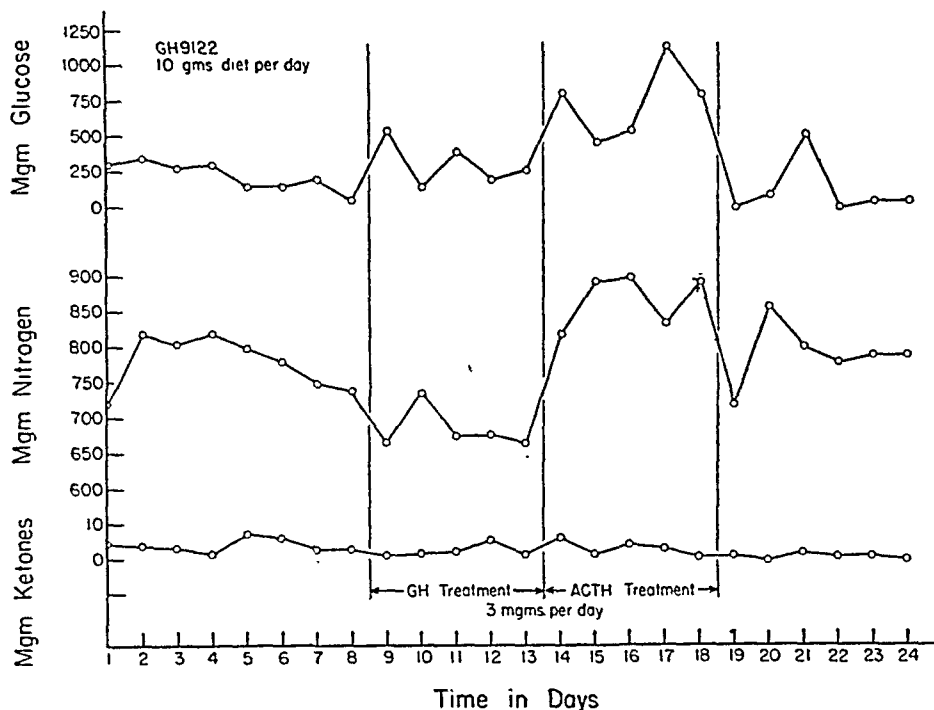


Fig. 1. Effect of GH and ACTH upon the urinary glucose, nitrogen and ketone bodies of a diabetic rat maintained on a carbohydrate-free diet.

reported (3), that the effect of adrenocorticotrophic hormone upon the glycosuria under these dietary conditions is less marked when the diabetes is severe. Also, in this

animal the adrenocorticotrophic hormone administration followed the period of growth hormone administration.

The data presented in table 2 show that in each instance in which growth hormone was administered there was a reduction in the excretion of urinary nitrogen. This reduction was significant in six of the seven cases in which statistical comparison was made. Figure 2 shows a similar effect of growth hormone in the rat that had its nitrogen excretion enhanced by prior administration of adrenocorticotrophic hormone. In each instance in which adrenocorticotrophic hormone was given there was an increase in urinary nitrogen excretion which was significant in at least one comparison

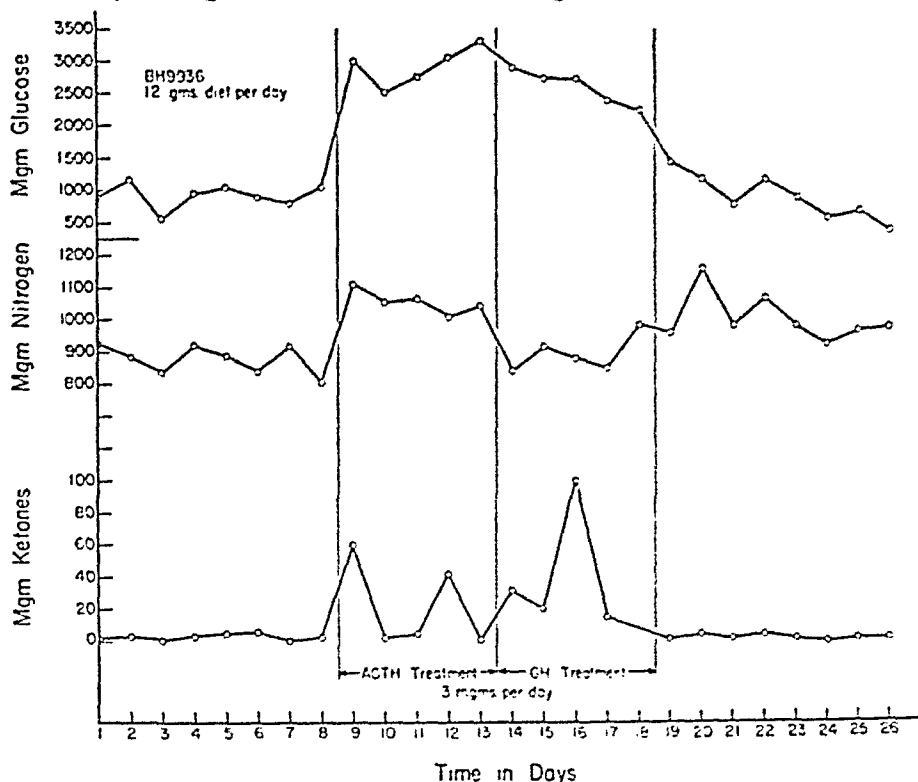


Fig. 2. Effect of GH and ACTH upon the urinary glucose, nitrogen and ketone bodies of a diabetic rat maintained on a carbohydrate-free diet.

in 4 of the 5 animals. It is clear, therefore, that under these experimental conditions the effect of adrenocorticotrophic hormone is to increase both the urinary nitrogen excretion and the glycosuria. On the other hand, there seems to be little doubt that growth hormone promotes nitrogen retention and is without effect upon the glycosuria.

Both hormones increased the ketonuria of the rats that were severely diabetic. The statistical summary is presented in table 3 and the variable nature of the response is shown in figures 1 and 2. Growth hormone produced an increase in ketonuria in three of the five experiments. Using the experiment numbers, cross reference to tables 1 and 2 shows that the increase in ketonuria occurred in those animals that had a marked glycosuria and D/N ratios above 2.69 during the injection period, rather than in the mildly diabetic rats. Adrenocorticotrophic hormone produced an

increase in ketonuria in four of six experiments. Again, this occurred only in animals with a marked glycosuria and high D/N ratios. Since both adrenocorticotrophic hormone and growth hormone will increase the ketonuria of fasted normal rats (2), it is not surprising that they have a similar effect in diabetic rats maintained on a carbohydrate-free diet. However, the above data would seem to indicate that the increased ketonuria is present only if the animal is severely diabetic.

#### CONCLUSIONS

Adrenocorticotrophic hormone enhances both the glycosuria and urinary nitrogen excretion of diabetic rats maintained on a carbohydrate-free diet. Growth hormone produces nitrogen retention but does not significantly alter the glycosuria of diabetic rats maintained on a carbohydrate-free diet. If the diabetes is severe, both growth hormone and adrenocorticotrophic hormone increase the ketonuria of diabetic rats maintained on a carbohydrate-free diet.

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# EFFECTS OF THE PITUITARY GROWTH AND ADRENOCORTICOTROPIC HORMONES ON THE URINARY GLUCOSE AND NITROGEN OF HYPOPHYSECTOMIZED DIABETIC RATS<sup>1</sup>

LESLIE L. BENNETT

*From the Division of Physiology and the Institute of Experimental Biology,  
University of California*

BERKELEY, CALIFORNIA

**T**HIS paper is the last of a series reporting studies of the metabolic effects of the pituitary growth and adrenocorticotrophic hormones in diabetic rats. Previous papers have reported experiments involving the administration of these hormones to diabetic rats maintained on a stock diet (1) and on a carbohydrate-free diet (2, 3).

It is the purpose of the present paper to report effects produced in hypophysectomized-diabetic rats by the pure growth and adrenocorticotrophic hormones.

## METHODS

The hormones used<sup>2</sup> were prepared according to the previously published methods (4, 5). The age and sex of animals used, the method of production of diabetes and the analytical methods for the determination of the daily urinary excretion of glucose and nitrogen were the same as those previously described (1). Animals with a persistent, stable degree of glycosuria were selected for study and were kept in individual metabolism cages.

Two different experiments were carried out: the first, an investigation of the effects of adrenocorticotrophic hormone; the second, an investigation of the effects of growth hormone. In each experiment there was a group of hypophysectomized-diabetic rats and a control group of intact diabetic rats. The control group in each case was pair-fed<sup>3</sup> with the corresponding experimental group. The food consumption was restricted prior to hypophysectomy; after hypophysectomy the hypophysectomized rats were allowed to eat *ad libitum*. In each group there were 4 or 5 animals that were carried through the entire period of 26 days of observation. The original number of animals in the groups were larger, but deaths after hypophysectomy, incomplete hypophysectomy and failure to maintain a stable daily food intake reduced the number of animals.

For seven days prior to hypophysectomy the daily urinary excretion of glucose and nitrogen was determined for a pre-hypophysectomy control period. After hypophysectomy the same determinations were made for a seven-day posthypophysectomy control period. The hormones were given for a period of four days and were administered intraperitoneally at a dose of one mg. three times a day. Following the period of hormone administration a post-injection control period of from five to six days was carried out.

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Received for publication July 19, 1948.

<sup>1</sup> Aided by grants from the American Cancer Society (through the National Research Council, Committee on Growth), the National Institute of Health RG-409 and the Research Board, University of California, Berkeley.

<sup>2</sup> The author is indebted to Dr. Choh Hao Li for preparation of the hormones used.

<sup>3</sup> The diet fed consisted of ground whole wheat 67.5%, casein 15.0%, whole milk powder 10.0%, NaCl 0.75%, CaCO<sub>3</sub> 1.5%, hydrogenated vegetable oil (Crisco or Primex) 5.25%. To each kg. of diet were added 3.5 gm. Sardilene (fish oil concentrate containing 3000 USP units of vitamin A and 400 chick units of vitamin D per gm.).

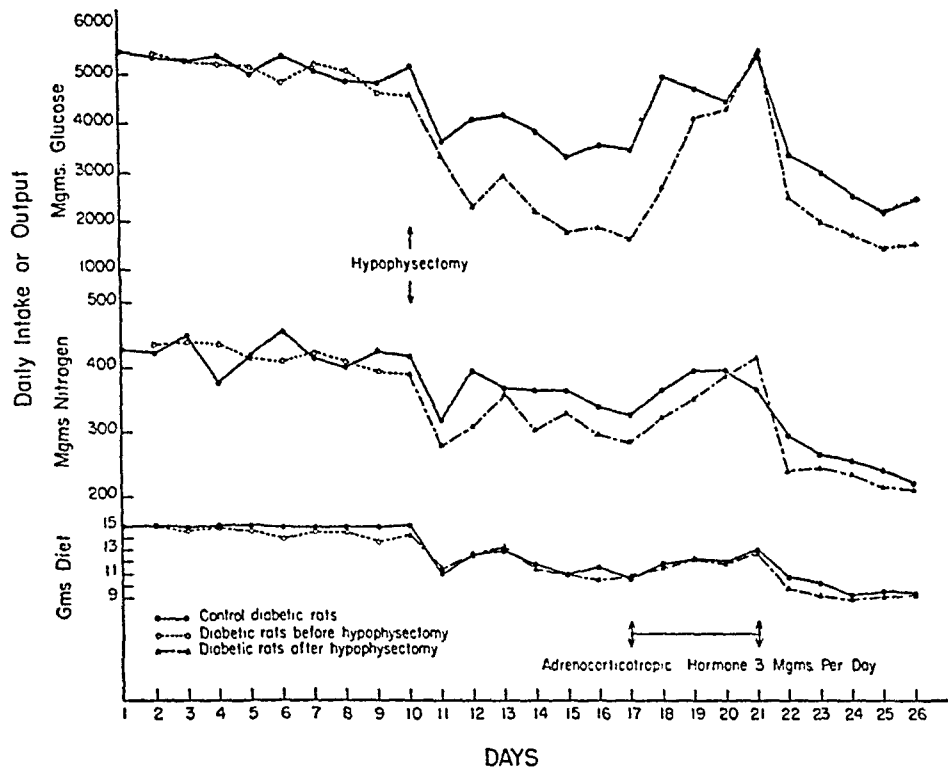


Fig. 1. DAILY FOOD CONSUMPTION, urinary nitrogen and urinary glucose of hypophysectomized diabetic rats treated with adrenocorticotrophic hormone.

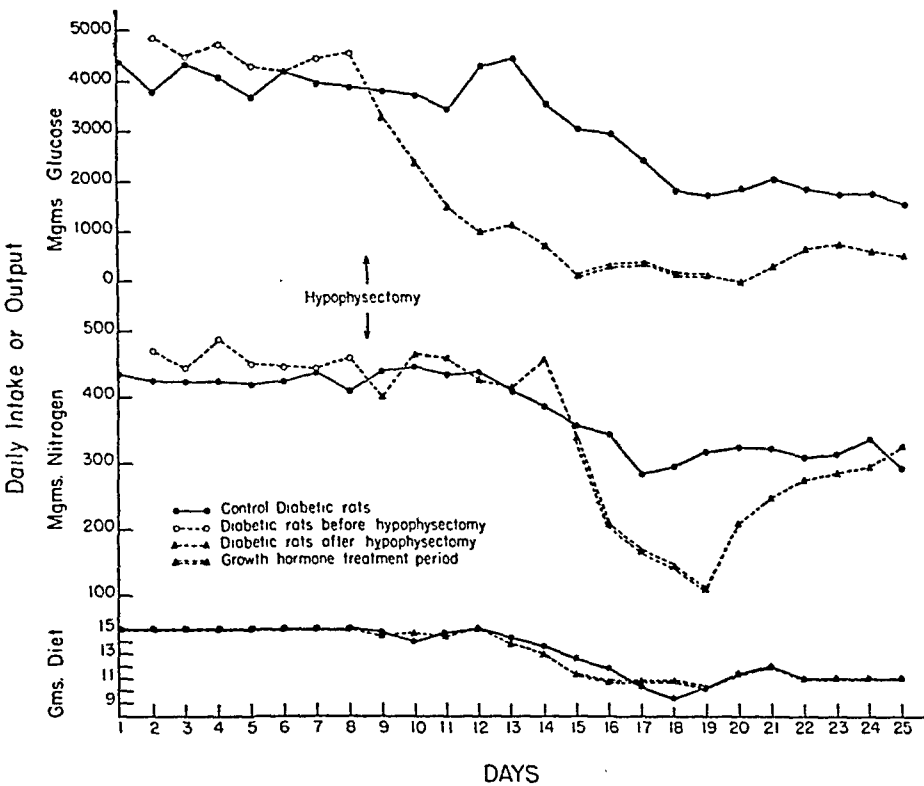


Fig. 2. DAILY FOOD CONSUMPTION, urinary nitrogen and urinary glucose of hypophysectomized diabetic rats treated with growth hormone.

## RESULTS AND COMMENT

The data from the experiments are presented in figures 1 and 2 and in table 1. In the graphic presentation is shown the average daily food consumption and urinary excretion of nitrogen and glucose by the group. In table 1 the average of individual hypophysectomized-diabetic rats during the injection period is compared with a control period. Inspection of figures 1 and 2 shows that prior to hypophysectomy the food consumption was quite constant and nearly identical in both the experimental and control groups and that there was essentially no difference in the urinary glucose and nitrogen excretion of the experimental group as compared to the control.

TABLE 1. EFFECT OF 3 MG. PER DAY OF ADRENOCORTICOTROPIC HORMONE AND GROWTH HORMONE ON THE URINARY GLUCOSE AND NITROGEN OF HYPOPHYSECTOMIZED-DIABETIC RATS

HORMONE GIVEN	FOOD INTAKE		URINARY GLUCOSE				URINARY NITROGEN			
	Control period <sup>1</sup>	Injection period	Control period	Injection period	Change	p	Control period	Injection period	Change	p <sup>2</sup>
	gm/day	gm/day	mg/day	mg/day	mg/day		mg/day	mg/day	mg/day	
ACTH	10.0	9.9	1460 ± 190 <sup>3</sup>	3010 ± 770	+1550	.03	273 ± 7	330 ± 11	+57	<.01
ACTH	8.7	10.8	930 ± 190	3390 ± 920	+2460	<.01	262 ± 31	356 ± 28	+106	.02
ACTH	10.6	10.7	2990 ± 450	4550 ± 320	+1560	.015	304 ± 24	333 ± 22	+29	.30
ACTH	13.3	14.9	2310 ± 370	4530 ± 630	+2220	<.01	360 ± 15	423 ± 26	+83	<.01
ACTH	13.1	13.2	2860 ± 320	4890 ± 890	+2030	.02	389 ± 31	400 ± 40	+11	.90
GH	11.2	12.1	380 ± 100	660 ± 160	+280	.10	312 ± 17	218 ± 36	-94	.02
GH	11.2	9.5	430 ± 115	220 ± 85	-210	.15	271 ± 22	154 ± 28	-117	<.01
GH	11.2	11.3	120 ± 50	70 ± 20	-50	.30	271 ± 14	160 ± 30	-111	<.01
GH	11.2	10.0	1610 ± 190	120 ± 30	-1490	<.01	297 ± 18	102 ± 8	-195	<.01

<sup>1</sup> For the ACTH-treated animals the last 5 days of the post-hypophysectomy control period was used. For the GH-treated animals the last 5 days of the post-injection control period was used. These control periods were taken as they gave the most uniform food intake compared with the injection period.

<sup>2</sup> From Fisher's table of *t*. A value of 0.05 or less was considered significant.

<sup>3</sup> Standard deviation of the mean.

After hypophysectomy, the hypophysectomized rats exhibited a marked reduction in glycosuria which was greater than could be accounted for by the reduced food intake, since a comparable reduction was not shown by the controls. The urinary nitrogen excretion<sup>4</sup> of both experimental and control groups was reduced slightly and to a comparable degree, seemingly in proportion to the reduction in food intake.

Adrenocorticotrophic hormone was administered both to the hypophysectomized-diabetic rats and to their unhypophysectomized controls. In both groups and in all animals there was a striking and significant increase in glycosuria, reaching its peak on the last day of injection. The increase in glycosuria could not be accounted for by an increase in food intake. In both groups there was a definite but not as strik-

<sup>4</sup> Unpublished observations of fecal nitrogen content have shown that it is not influenced by hypophysectomy nor by growth or adrenocorticotrophic hormone administration. Thus, they are not reported in these experiments.

ing or consistent an increase in urinary nitrogen. Thus, previous work regarding the diabetes-enhancing effect of crude adrenocorticotrophic preparations (6) has been fully confirmed with the pure hormone. It increases both the glycosuria and urinary nitrogen excretion of diabetic rats. Adrenocorticotrophic hormone may also be said to be diabetes-enhancing with respect to the ketonuria of diabetic animals (3), since under appropriate experimental conditions it is increased as well. In these experiments as in the previous ones in which animals were maintained on the stock diet (1), the increased glucose in the urine could not have been derived from the additional protein broken down under the influence of the hormone.

Growth hormone was administered only to the hypophysectomized-diabetic rats, not to their controls. In no case was there produced a significant increase in glycosuria. In fact, in three cases the glycosuria decreased. In every case growth hormone produced nitrogen retention, with its effect apparently persisting for at least 24 hours after the cessation of its injection. Thus, in no sense can growth hormone be considered diabetes-enhancing with respect to its effect upon the protein metabolism of diabetic rats. It is doubtful if growth hormone can be considered diabetes-enhancing with respect to its effect upon the glycosuria of diabetic rats since it was without effect in the present experiment; it was without effect in diabetic rats maintained on a carbohydrate-free high-protein diet (3) and it only occasionally increased the glycosuria of rats on the stock diet (1). However, growth hormone may be said to be diabetes-enhancing with respect to the ketonuria of diabetic rats, since it increased the ketonuria of diabetic rats fed a carbohydrate-free high-protein diet (3).

#### CONCLUSIONS

Adrenocorticotrophic hormone increases both the urinary glucose and nitrogen excretion of hypophysectomized-diabetic rats. Growth hormone is without effect upon the urinary glucose but reduces the urinary nitrogen excretion of hypophysectomized-diabetic rats.

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# EFFECT OF PARENTERAL ADMINISTRATION OF GLUCOSE AND PROTEIN HYDROLYSATE ON FOOD INTAKE IN THE RAT

HENRY JANOWITZ AND M. I. GROSSMAN

*From the Department of Clinical Science, University of Illinois College of Medicine*

CHICAGO, ILLINOIS

ONE of the most remarkable adjustments which animals and men make is the regulation of food intake in accordance with bodily needs. The fact that most animals show a high constancy in body weight, even when large variations in caloric expenditure occur, indicates that food intake is normally closely attuned to caloric need.

An analysis of the factors concerned in this regulation is the study of hunger and appetite.

The simplest form that this regulatory mechanism could take would be for each unit expenditure of energy to reflect itself in a unit drive to eat. The resultant intake of a unit quantity of food of unit potential energy would then balance the existing caloric deficit and restore caloric equilibrium. Variation of expenditure of energy, and potential energy of available food stuffs, would be directly reflected in variation of intake of food.

If such a regulatory mechanism were sensitive to relatively small caloric deficits, it would tend to restore these deficits from meal to meal, or from day to day, depending on the eating habits of the species involved.

In this theoretical manner, caloric expenditure and food intake would be nicely balanced at a rapid rate with constancy of body weight.

It would also follow that supplying the animal economy with units of potentially available energy in the form of parenteral administration of calorically valuable material would result in the rapid restoration of caloric equilibrium by the diminution of food intake by comparable units.

The experiments reported in this paper were designed to test this hypothesis by noting the effects of intraperitoneal injection of glucose and protein hydrolysate mixtures upon the food intake of the rat.

## METHODS

Ten male white rats grown in this laboratory ranging in weight from 180 to 300 gm. were housed in individual cages in a relatively constant temperature room, with free access to water at all times.

The animals were divided into two groups of 5 rats each, according to their basal diet. The first group, series A, was maintained on a fluid diet containing 5 per cent glucose and 5 per cent protein hydrolysate (amigen). The fluid diet was given by the conventional drinking bottle. It supplied 0.35 cal/cc. A daily vitamin supplement in liquid form consisting of thiamine hydrochloride 20  $\mu$ g, riboflavin 25  $\mu$ g, pyridoxine 20  $\mu$ g, calcium pantothenate 100  $\mu$ g and nicotinic acid 100  $\mu$ g was fed by mouth.

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Received for publication July 26, 1948.

The second group of animals, series B, was maintained on an adequate commercial dry food (Purina, Chow) and supplemented by the vitamin mixture as above. The chow was supplied in a small glass jar placed within a deeper jar and thereby scattering was held to a minimum. This diet supplied 3.3 cal/gm.

The 24-hour intake of food was measured daily at the same hour. The animals were weighed daily, and all gained weight during the period of observation.

The solutions used for intraperitoneal injection were 1) 0.5 per cent NaCl as a control, 2) 10 per cent glucose in 0.3 per cent NaCl and 3) 5 per cent glucose-5 per cent amigen mixture. On treatment days one of these solutions was injected two times during the day, 15 cc. each time, except in one animal where 20 cc. were injected each time. The order of administration of the solutions was randomized and a day of rest intervened between each day of treatment. Each test solution was used in each rat on three to seven test days.

## RESULTS

### *Series A. Rats on Liquid Diet*

From the results summarized in table 1 it is readily apparent that some depression of food intake occurred in all treatment periods, including those in which saline alone was administered. The average reduction in food intake associated with saline injection was as large as that observed with the injection of foodstuffs. The range of decrement of caloric intake attending the injection of 30 cc. of 5 per cent glucose-5 per cent amigen (from 3.5 cal/day to 10.85 cal/day) and of 30 cc. of 10 per cent glucose (from 1.05 cal/day to 10.2 cal/day) did not exceed that produced by 30 cc. of 0.5 per cent saline (from 3.15 cal/day to 16.7 cal/day). In only 2 rats did the average depression of food intake by both glucose and glucose-amigen mixtures exceed that produced by saline. Further, only in *rat A<sub>3</sub>* did the average depression of calories ingested equal or exceed the caloric value of the injected material.

### *Series B. Rats on Stock Diet*

The results summarized in table 2 indicate that although some depression of average food intake occurred in most instances, this was not a constant finding. In one instance no depression took place and in 2 animals a slight increase in food intake was noted. In 4 animals depressions of average food intake were induced by saline as well as by the nutrient materials. The range of change induced by 30 cc. of 5 per cent amigen-5 per cent glucose (from 0 to -8.6 cal/day) and by 30 cc. of 10 per cent glucose in 0.3 per cent saline (from +0.3 cal/day to -7.3 cal/day) did not exceed the range of changes induced by 30 cc. of 0.5 per cent NaCl (from +3 cal/day to -11.2 cal/day).

In no animal did the depression of food intake by both glucose and glucose + amigen mixtures exceed that produced by saline. Further, only in *rat B<sub>2</sub>* did the depression of calories ingested equal or exceed the caloric value of the material injected. None of the variations in the degree of depression, produced by the various intraperitoneal injections, are of sufficient magnitude to be significant.

## DISCUSSIONS

These experiments indicate that the depression of food ingestion induced in rats by the intraperitoneal injection of 10 per cent glucose and 5 per cent glucose + 5 per cent amigen mixtures is of the same order of magnitude as that induced by injection

of comparable volumes of saline, and that this decrement is not related to the caloric value of the materials injected. Within the periods of this experiment administration of from 17 to 25 per cent of the daily caloric requirement of the rat did not diminish food intake beyond that produced by an equal volume of non-caloric material.

These findings are in keeping with the failure of intravenous glucose to inhibit

TABLE 1

ANIMAL NO.	PERIOD	VOL. GIVEN I.P.	NO. OF DAYS	CAL. GIVEN I.P.	VOL. OF DIET INGESTED DAILY	AVERAGE CAL. INGESTED DAILY	CHANGE FROM CONTROL PERIOD
		cc.			cc.		Cal/day
A <sub>1</sub>	Control		24		175	61.35	
	NaCl <sup>1</sup>	30	5	0	154	53.9	-7.4
	Amigen <sup>2</sup>	30	5	10.5	152	53.2	-8.1
	10% glucose <sup>3</sup>	30	5	12	146	51.1	-10.2
A <sub>2</sub>	Control		18		171	59.85	
	NaCl	30	4	0	125	43.75	-16.7
	Amigen	30	4	10.5	161	56.35	-3.5
	10% glucose	30	5	12	145	50.75	-9.1
A <sub>3</sub>	Control		17		173	60.55	
	NaCl	30	6	0	156	54.60	-5.95
	Amigen	30	6	10.5	142	49.70	-10.85
	10% glucose	30	6	12	166	58.1	2.45
A <sub>4</sub>	Control		15		168	58.80	
	NaCl	30	4	0	157	54.95	-3.85
	Amigen	30	5	10.5	147	51.45	-7.35
	10% glucose	30	5	12	150	52.5	-6.30
A <sub>5</sub>	Control		21		173	60.55	
	NaCl	30	4	0	164	57.40	-3.15
	Amigen	30	3	10.5	154	53.90	-6.60
	10% glucose	30	3	12	170	59.50	-1.05

<sup>1</sup> 0.5% NaCl. <sup>2</sup> 5% glucose-5% protein hydrolysate (amigen). <sup>3</sup> 10% glucose in 0.3% NaCl.

food intake in the dog (1), and with the previously reported failure of intravenously administered fat emulsions in the dog (2), and certain amino acid mixtures in man (3) to inhibit food intake in short-term experiments. This interpretation is at variance with that recently reported by Adolph (4). This observer noted that injected nutrients reduced the voluntary intake of food by mouth in the rat and concluded that "Plethora inhibits eating . . . even when the alimentary tract has not been concerned in the creation of the plethora". However it should be noted that the effects of injection of non-caloric material were not studied and detailed protocols were not furnished.

The results detailed in this study do not support the hypothesis of a simple regulatory mechanism outlined in the introduction above, in which variation of caloric requirement would be easily and rapidly balanced by variation in food intake, from meal to meal, or from day to day.

Caloric requirements, decreased by the parenteral administration of calorically

TABLE 2

ANIMAL NO.	PERIOD	VOL. GIVEN I.P.	NO. OF DAYS	CAL. GIVEN I.P.	AVERAGE CAL. EATEN DAILY	CHANGE FROM CONTROL PERIOD
		cc.				Cal/day
<i>B</i> <sub>1</sub>	Control		28		61.7	
	NaCl <sup>1</sup>	30	7	0	53.4	-8.3
	Amigen <sup>2</sup>	30	7	10.5	53.1	-8.6
	10% glucose <sup>3</sup>	30	7	12	54.8	-6.9
<i>B</i> <sub>2</sub>	Control		21		78.3	
	NaCl	40	4	0	49.5	-28.7
	Amigen	40	4	14	53.4	-24.8
	10% glucose	40	4	16	51.8	-26.4
<i>B</i> <sub>3</sub>	Control		27		45.5	
	NaCl	30	5	0	34.3	-11.2
	Amigen	30	5	10.5	40.2	-5.3
	10% glucose	30	5	12	38.2	-7.3
<i>B</i> <sub>4</sub>	Control		25		49.8	
	NaCl	30	6	0	52.8	+3
	Amigen	30	7	10.5	49.8	0
	10% glucose	30	7	12	49.2	-1.6
<i>B</i> <sub>5</sub>	Control		22		51.2	
	NaCl	30	6	0	43.3	-7.9
	Amigen	30	6	10.5	47.2	-4.0
	10% glucose	30	6	12	51.5	+0.3

<sup>1</sup> 0.5% NaCl.    <sup>2</sup> 5% glucose-5% protein hydrolysate (amigen).    <sup>3</sup> 10% glucose in 0.3% NaCl.

valuable material, were not balanced by corresponding decrement of food intake as measured from day to day.

The regulatory mechanism would seem either not to be sensitive to variations of caloric requirement within this range (up to 25%), or else to respond to these variations at a much slower rate than that presupposed by any day to day, or meal to meal readjustment. In view of the precision which normally occurs in adjustment of food intake to energy expenditure, and the high constancy of body weight, it appears most likely that such adjustments are not made from meal to meal, or from day to day, but require longer periods of time than those available to the animal in this set of experiments.



## SUMMARY AND CONCLUSIONS

The intraperitoneal administration of glucose and protein hydrolysate mixtures to the rat in calorically significant amounts did not produce an equivalent depression of food intake. The depression which did occur was not greater than that produced by saline injections. The normal regulatory mechanisms responsible for the precise adjustment of food intake to caloric requirement are not operative during the short period of these experiments.

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# A DIETARY FACTOR REGULATING THE ENZYME CONTENT OF THE PANCREAS: CHANGES INDUCED IN SIZE AND PROTEOLYTIC ACTIVITY OF THE CHICK PANCREAS BY THE INGESTION OF RAW SOY-BEAN MEAL<sup>1</sup>

S. S. CHERNICK, S. LEPKOVSKY AND I. L. CHAIKOFF

*From the Division of Physiology of the Medical School and the Division of Poultry Husbandry of the College of Agriculture, University of California*

BERKELEY, CALIFORNIA

IT IS shown in the present communication that the feeding of raw soy-bean meal induces profound changes in the chick pancreas not only in its size but also in its trypsin content. This finding is of particular interest, for the failure of raw soy-bean meal to support maximal growth has been ascribed to a trypsin inhibitor (1), which has been isolated from the bean in crystalline form (2). Concentrates of this inhibitor retard growth even when added to diets containing nutritionally adequate animal proteins (1, 3). On the basis of the findings presented here it is postulated that the acinar tissue of the pancreas is stimulated to increased activity by the presence of dietary proteolytic inhibitors in the intestinal tract.

## EXPERIMENTAL

### *Treatment of Animals*

The White Leghorn chicks used in each experiment were obtained from a single hatch. They were kept in electrically heated battery-brooders. For the first week after hatching they were fed a 'starter' ration<sup>2</sup>; thereafter groups of these chicks whose weights were in close agreement were fed soy-bean diets. Raw or heated soy-bean meal served as the source of protein in these diets. The beans were ground in a corn mill at room temperature. The *cooked* soy beans were prepared by heating the ground beans in an autoclave at one-pound pressure for 45 minutes. The raw and cooked soy-bean diets used in each experiment were prepared from a single batch of beans. Since the batches of soy beans varied somewhat in their protein contents, the desired protein composition of each diet was obtained by varying the proportions of soy-bean meal and cerelese. A typical example of the composition of the diets used in this investigation is recorded in table 1.

Chicks were sacrificed by dislocation of the cervical vertebra. Their entire

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Received for publication July 26, 1948.

<sup>1</sup> This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces. The views and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of the Army.

<sup>2</sup> The starter ration had the following composition: corn, 300 parts; wheat, 135; barley, 100; alfalfa meal, 40; wheat bran, 150; sardine meal, 75; soy-bean meal, 100; dried whey, 25; dried skim milk, 25; liver meal, 20; oyster shell, 15; bone meal, 12; salt, 5; manganese sulfate, 0.5; Sardilene (a fish oil containing 400 units of Vitamin D and 3000 units of Vitamin A per cc.), 2.5 parts. To each 1000 lbs. of the above mixture, one gram of riboflavin was added.

pancreas were rapidly removed, freed of extraneous tissue, immediately chilled, and weighed. Various other organs were also excised and weighed.

The chilled pancreases were transferred to a tube (kept cold by immersion in an ice-salt bath) and homogenized with a few cc. of ice-cold distilled water. The homogenate was then diluted with distilled water to yield a concentration of about 20 mg. of tissue per cc. Unless analyses of the homogenate were carried out immediately, it was stored at  $-15^{\circ}\text{C}$ . No conversion of trypsinogen to trypsin occurred under these conditions, since proteolytic activity of homogenates was found to be negligible in the absence of added enterokinase.

#### *Determination of Proteolytic Activity of Pancreas Homogenates*

As stated above, pancreas homogenates do not digest proteins in the absence of enterokinase. For example, when as much as 5 mg. of chick pancreas was added to denatured hemoglobin substrate (see below), no detectable proteolysis occurred. After incubation with enterokinase, however, as little as 0.05 mg. of pancreas digested measurable quantities of hemoglobin. The increase in the proteolytic power

TABLE I. COMPOSITION OF TYPICAL DIETS (EXPERIMENT 3)

DIETS	SOY BEAN MEAL	CEREOSE	YEAST	VITAMIN-SALT SUPPLEMENT <sup>1</sup>
	%	%	%	
10% protein.....	24.9	65.85	5	4.25
25% protein.....	62.3	28.45	5	4.25

<sup>1</sup> This supplement had the following composition: Sardilene (a fish oil containing 400 units of vitamin D and 3000 units of vitamin A per cc.) 6%,  $\text{CaCO}_3$  24%,  $\text{Ca}^3(\text{PO}_4)_2$  47%, salt mixture 23%, riboflavin 0.2 mg. per 100 gm. of mixture.

of the homogenate is due to conversion of the proenzymes (trypsinogen and chymotrypsinogen) to active proteinases (trypsin and chymotrypsin). The conditions which favor this conversion have been extensively studied by Kunitz (4). On the basis of his findings preliminary experiments were performed to determine the optimal conditions for activation of the proteolytic activity of pancreas homogenates. Such factors as temperature,  $p\text{H}$ , concentration of enterokinase, concentration of pancreas, and period of incubation were studied. The following method was then developed for determination of proteolytic activity of the pancreas homogenates.

*Activation of Pancreatic Proteinases by Enterokinase.* Purified enterokinase was prepared from the duodenal mucosa of dogs by the method of Kunitz (4). The material derived from one gm. of mucosa was dissolved in one cc. of a 0.02M phosphate buffer ( $p\text{H}$  7.6). This solution was stored at  $-15^{\circ}$  and was used throughout the experiments reported here.

Before use the temperature of the enterokinase solution was raised to  $0^{\circ}$  and an aliquot of it diluted 1:50 with cold 0.02M phosphate buffer ( $p\text{H}$  5.8). The pancreas homogenates were also diluted with this same buffer so that each cc. contained about 2 mg. of pancreas. Five cc. of the diluted enterokinase solution, 2 to 5 cc. of the diluted pancreas suspension and sufficient 0.02M phosphate buffer ( $p\text{H}$  5.8) to make

a total volume of 15 cc. were transferred to each of several glass-stoppered Erlenmeyer flasks. The flasks were then placed in a constant temperature bath at 37.5°C. and shaken for 15 minutes. Periods of activation longer than 15 to 30 minutes were found to result in a gradual decline of the proteolytic activity of the mixture.

Immediately following its activation, the pancreas suspension was made up to a volume of either 25 or 50 cc. by the addition of cold 0.02M phosphate buffer (pH 7.6) and rapidly chilled to 0°. The suspension was kept at 0° until samples were taken for determination of its proteolytic activity (less than 1 hr. later). In some cases several aliquots were diluted with the pH 7.6 buffer to provide a range of proteolytic activities. As a rule, the samples of activated solution used for the determination of proteolytic activity contained 0.1 to 0.5 mg. of pancreas, the amount varying, of course, with the concentration of proteinases.

*Measurement of Proteolysis.* Anson's method (5) was employed for the determination of the proteolytic activity of the activated pancreas solutions. It involves the hydrolysis of denatured hemoglobin (prepared from beef corpuscles) and the measurement of its split products (largely peptides) which are soluble in trichloroacetic acid. The substrate solution contained about 2 per cent of the denatured hemoglobin in phosphate buffer (pH 7.6). One-cc. aliquots of the activated pancreas solution and 5 cc. of hemoglobin solution were incubated for 10 minutes at 37.5°. The reaction was terminated by the addition of 10 cc. of 0.3 N trichloroacetic acid. The filtrate obtained was alkalized and treated with the phenol reagent (5), the color was allowed to develop for 5 to 10 minutes and measured by a photoelectric colorimeter. A red filter (Klett-Summerson No. 66) was used. The color values for the substrate-pancreas mixtures at zero time were determined by adding enzyme solution to a mixture of the substrate and trichloroacetic acid.

Control determinations were performed to ascertain the color products resulting from autolysis of pancreas, digestion of hemoglobin by enterokinase alone, and non-enzymatic breakdown of hemoglobin. These corrections were very small and consequently were ignored without introducing an error greater than 5 per cent.

The unit of proteolytic activity is defined by Anson as "The amount which digests hemoglobin under the standard conditions at an initial rate such that there is liberated per minute an amount of split products not precipitated by trichloroacetic acid which gives the same color with the phenol reagent as one milliequivalent of tyrosine". The color values of standard tyrosine solutions were determined and compared with the values obtained by proteolysis. The trypsin units per gm. pancreas were then calculated. Trypsin units as used in this study imply measurement of proteolytic activity at pH 7.6. No attempt was made to distinguish between the proteolytic activities of trypsin and chymotrypsin.

The nitrogen content of the pancreases was determined by the semi-micro Kjeldahl method.

## RESULTS

### *Body and Organ Weights*

*Body and Pancreas Weights of Chicks Fed Raw and Cooked Soy-Bean Protein Diets.* In *experiment 1* all birds were killed at three weeks of age, and during the

last two weeks they were fed diets containing 10 to 25 per cent raw or autoclaved soy-bean protein (table 2). A comparison of the weights of birds fed the four levels of soy-bean protein brings out again the effect of heating on the biological value of this protein. This is particularly evident at the higher levels of intake. Thus, in *experiment 1*, the birds fed a diet containing 20 per cent raw soy-bean protein weighed

TABLE 2. INFLUENCE OF SOY BEAN DIETS ON THE SIZE AND ENZYME CONTENT OF THE PANCREAS

EXP.	NO. OF CHICKS USED	PERIOD ON DIET <sup>1</sup>	CHICK WT. <sup>2</sup>	PROTEIN CONTENT OF DIET	CONDITION OF SOY BEAN MEAL	PANCREAS <sup>3</sup>			
						Total wet wt.	Per cent of body wt.	Proteolytic activity <sup>4</sup>	
								Per gm. fresh pancreas	Per gm. pancreas nitrogen
		days	gm.	%		gm.		units <sup>4</sup>	units <sup>4</sup>
1 <sup>6</sup>	4	14	70	10	Raw	0.47	0.63	0.07	3.1
	4	14	80	10	Cooked <sup>5</sup>	0.33	0.42	0.07	2.5
	3	14	65	15	Raw	0.33	0.55	0.23	8.6
	4	14	145	15	Cooked	0.61	0.42	0.15	5.5
	4	14	81	20	Raw	0.54	0.65	0.20	7.0
	4	14	149	20	Cooked	0.65	0.45	0.13	5.0
	4	14	106	25	Raw	1.02	0.95	0.29	10.3
	4	14	167	25	Cooked	0.75	0.44	0.27	8.6
2 <sup>6</sup>	3	23	91	10	Raw	0.66	0.74	0.25	10.1
	5	23	115	10	Cooked	0.55	0.48	0.21	7.4
	5	23	116	18	Raw	0.80	0.66	0.26	9.3
	5	23	176	18	Cooked	0.77	0.44	0.19	6.7
	5	23	158	26	Raw	1.41	0.88	0.46	14.5
	5	23	265	26	Cooked	1.11	0.42	0.23	7.9
3 <sup>6</sup>	10	23	56	10	Raw	0.46	0.82	0.11	4.8
	10	23	92	10	Cooked	0.51	0.56	0.07	2.7
	10	23	116	25	Raw	1.21	1.04	0.38	11.7
	10	23	190	25	Cooked	0.89	0.47	0.18	6.1

<sup>1</sup> For the first week after hatching the chicks were fed a 'starter' ration; thereafter, they received the experimental diet. The chicks in *exp. 1* were 21 days old when killed; those in *exp. 2* and 3 were 30 days old when killed.

<sup>2</sup> Each value is the average of 3-10 closely agreeing results obtained from as many birds.

<sup>3</sup> A measure of both trypsin and chymotrypsin activity (see text).

<sup>4</sup> Trypsin units as defined by Anson (5). See text.

<sup>5</sup> Autoclaved at 1 lb. steam pressure for 45 min.

<sup>6</sup> These are the same experiments as those recorded in table 4.

81 gm. (average), whereas those that received the diet containing the same percentage of autoclaved protein weighed 149 gm. (average). The weight difference was equally striking in birds fed diets containing 26 per cent protein (*experiment 2*).

In chicks fed the autoclaved soy-bean diet, the amount of pancreas tissue per 100 gm. body weight remained quite constant. Thus in birds whose average weights varied from 80 to 167 gm. (*experiment 1*) the average pancreas values were 0.42,

0.42, 0.45, and 0.44 for the four groups that received respectively 10, 15, 20, and 25 per cent protein diets. The birds fed the same levels of protein derived from *raw* soy-beans weighed 65 to 106 gm. (averages) at three weeks of age; the average weights of their pancreases were respectively 0.63, 0.55, 0.65, and 0.95 gm. per 100 gm. body weight.

In *experiments 2* and *3* the birds were sacrificed at 30 days of age. The results obtained in these two experiments (table 2) show again that birds fed diets containing *raw* soy-beans as the sole source of protein have larger pancreases than those fed the autoclaved soy-diets.

In table 3 are recorded pancreas weights for birds fed a normal diet, namely the U.C. stock ration<sup>3</sup>. The average values did not exceed 0.56 per cent of their body weights. Latimer (6) also found that pancreases in chicks weighing between 50 and 250 gm. and fed a normal mixed diet amounted to about 0.5 per cent of their body

TABLE 3. ORGAN WEIGHTS AND PROTEOLYTIC ACTIVITY OF THE PANCREAS OF CHICKS FED THE U.C. STOCK RATION<sup>1</sup> (EACH VALUE IS THE AVERAGE OF 5-8 CLOSELY AGREEING RESULTS OBTAINED FROM AS MANY BIRDS)

NO. OF CHICKS USED	CHICK WT.	CHICK AGE	ORGAN WT. AS PERCENTAGE OF BODY WT.			TRYPTIC ACTIVITY/GM. PANCREAS NITROGEN
			Pancreas	Liver	Heart	
	gm.	days				units <sup>2</sup>
8	166	28	0.52			8.9
8	202	30	0.56	3.2	0.68	8.4
8	223	30	0.51			
5	220	30	0.47	2.7		7.5

<sup>1</sup> The diet had the following composition: corn, 300 parts; wheat, 135; barley, 100; alfalfa meal, 40; wheat bran, 150; sardine meal, 75; soy bean meal, 100; dried whey, 25; dried skim milk, 25; liver meal, 20; oyster shell, 15; bone meal, 12; salt, 5; manganese sulfate, 0.5; fish oil, 2.5. Riboflavin was added to the extent of 1 gm/1000 lbs. of diet.

<sup>2</sup> Trypsin units determined by the method of Anson (5).

weights. In view of these observations, it seems reasonable to conclude that hypertrophy of the pancreas had occurred in the birds fed the *raw* soy-bean meal.

*Liver and Heart Weights of Birds Fed Raw and Autoclaved Soy-Bean Diets.* That the increase in size of the pancreas in chicks fed a raw soy-bean diet is specific for only this organ is brought out in figures 1, 2, and 3. In these, organ weights were plotted against body weights. The data were obtained from *experiments 2* and *3*. In *experiment 3*, for example, the weights of the livers and hearts of chicks fed raw soy did not vary consistently from those fed autoclaved soy. Nor in *experiment 2* was there a consistent difference in the liver weights between the chicks fed raw and those fed autoclaved soy.

*Effect of Supplemental Methionine on Pancreas Size and Body Weight of Chicks.* Since methionine corrects to a large extent the deficiencies induced by a *raw* soy-bean diet (7), the effect of supplementing the raw soy-bean diets with this amino acid upon pancreas size was investigated. The results are recorded in table 4.

<sup>3</sup> Its composition is described in table 3.

The addition of 0.5 per cent methionine to the *raw* soy-bean diets, even when the protein contents of the diets were as high as 25 to 26 per cent, resulted in marked stimulation in the growth of the chicks. Thus 30-day old chicks fed the raw soy-bean diet containing 26 per cent protein for three weeks had an average weight of 158 gm. (*experiment 2*); those fed the same diet plus 0.5 per cent methionine weighed 267 gm. (average).

TABLE 4. EFFECT OF SUPPLEMENTAL METHIONINE ON THE SIZE AND ENZYME CONTENT OF THE PANCREAS OF CHICKS FED SOY BEAN DIETS

SOY BEAN MEAL	EXP. <sup>4</sup>	NO. OF CHICKS USED	PERIOD ON DIET <sup>1</sup>	CHICK WT. <sup>2</sup>	PROTEIN CONTENT OF DIET	METHIONINE ADDED	PANCREAS <sup>3</sup>			
							Total wet wt.	Per cent of body wt.	Proteolytic activity <sup>1</sup>	
									Per gm. fresh pancreas	Per gm. pancreas nitrogen
			<i>days</i>	<i>gm.</i>	<i>%</i>	<i>%</i>	<i>gm.</i>	<i>gm.</i>	<i>units<sup>4</sup></i>	<i>units<sup>4</sup></i>
Raw	1	4	14	106	25	None	1.02	0.95	0.29	10.3
		4	14	146	25	0.5	1.49	1.03	0.37	10.9
	2	5	23	158	26	None	1.41	0.88	0.46	14.5
		5	23	267	26	0.5	2.33	0.87	0.34	10.4
	3	10	23	116	25	None	1.21	1.04	0.38	11.7
		10	23	163	25	0.5	1.77	1.05	0.46	13.1
Cooked <sup>5</sup>	1	4	14	167	25	None	0.75	0.44	0.27	8.6
		4	14	175	25	0.5	0.70	0.40	0.15	4.8
	2	5	23	265	26	None	1.11	0.42	0.23	7.9
		5	23	258	26	0.5	1.02	0.39	0.18	5.8
	3	10	23	190	25	None	0.89	0.47	0.18	6.1
		10	23	206	25	0.5	0.95	0.46	0.22	7.1

<sup>1</sup> For the first week after hatching chicks were fed a 'starter' ration, thereafter they received the experimental diets recorded in table 1.

<sup>2</sup> Each value is the average of ~10 closely agreeing results obtained from as many birds.

<sup>3</sup> A measure of both trypsin and chymotrypsin activity (see text).

<sup>4</sup> Trypsin units as defined by Anson (5).

<sup>5</sup> Autoclaved at 1 lb. steam pressure for 45 min.

<sup>6</sup> These are the same experiments as those recorded in table 2.

The increase in body weight produced by supplementing the raw soy-bean diet with 0.5 per cent methionine was accompanied by an increase in pancreas size, but the ratio of pancreas weight to body weight was *not* changed; in both groups of birds the pancreas weights amounted to 0.9 to 1.0 per cent of their body weights.

The addition of methionine to diets containing 25 or 26 per cent autoclaved soy-bean protein as the source of protein failed to increase the body weights of the chicks. The pancreas weights of the birds fed these same levels of *autoclaved* soy

protein with or without supplemental methionine did not exceed 0.5 per cent of their body weights.

### *Proteolytic Activity of Pancreas*

*Proteolytic Activity of Pancreases of Chicks Fed Raw and Autoclaved Soy Beans.* Values for proteolytic activity of the pancreas as determined by the procedure of

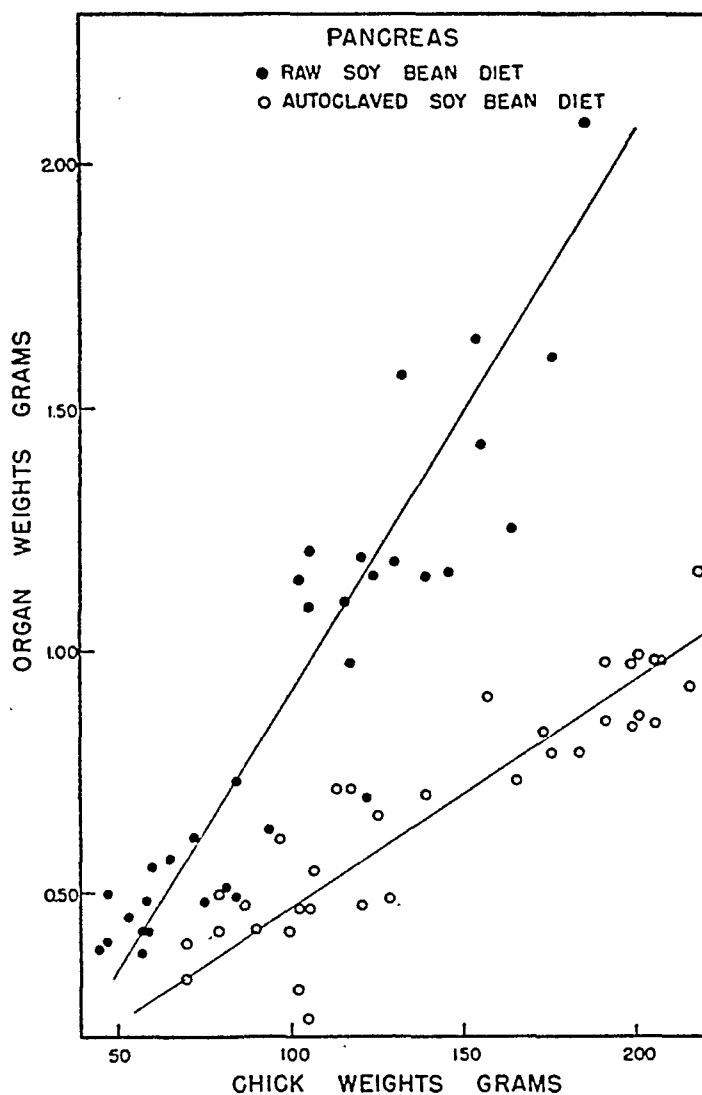


Fig. 1. PANCREAS WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets. The data were obtained from *experiments 2 and 3*.

Anson are recorded in table 2. As already pointed out, this method measures combined tryptic and chymotryptic proteolysis. The tryptic content expressed as either per gm. of fresh pancreas or per gm. of nitrogen was greater in birds fed raw than in those fed autoclaved soy-bean meal. Thus the pancreases of the chicks of *experiment 3*, which were fed a raw soy-bean diet providing 25 per cent protein, contained 11.7 trypsin units per gm. of nitrogen (average value), whereas those fed a diet with the same level of autoclaved soy-bean protein contained an average of 6.1 units per gm.



nitrogen. The differences in the proteolytic contents of the pancreases between birds fed raw and those fed autoclaved soy-bean diets were equally striking when the results were compared in terms of the whole gland or per kilo of body weight.

*Effect of Supplementing Soy-Bean Diets with Methionine upon Proteolytic Activity of Chick Pancreas.* Although supplemental methionine increased the weights of chicks fed raw soy beans at the 25 per cent protein level, table 4 shows that it did not change the concentration of proteolytic enzymes in the pancreas.

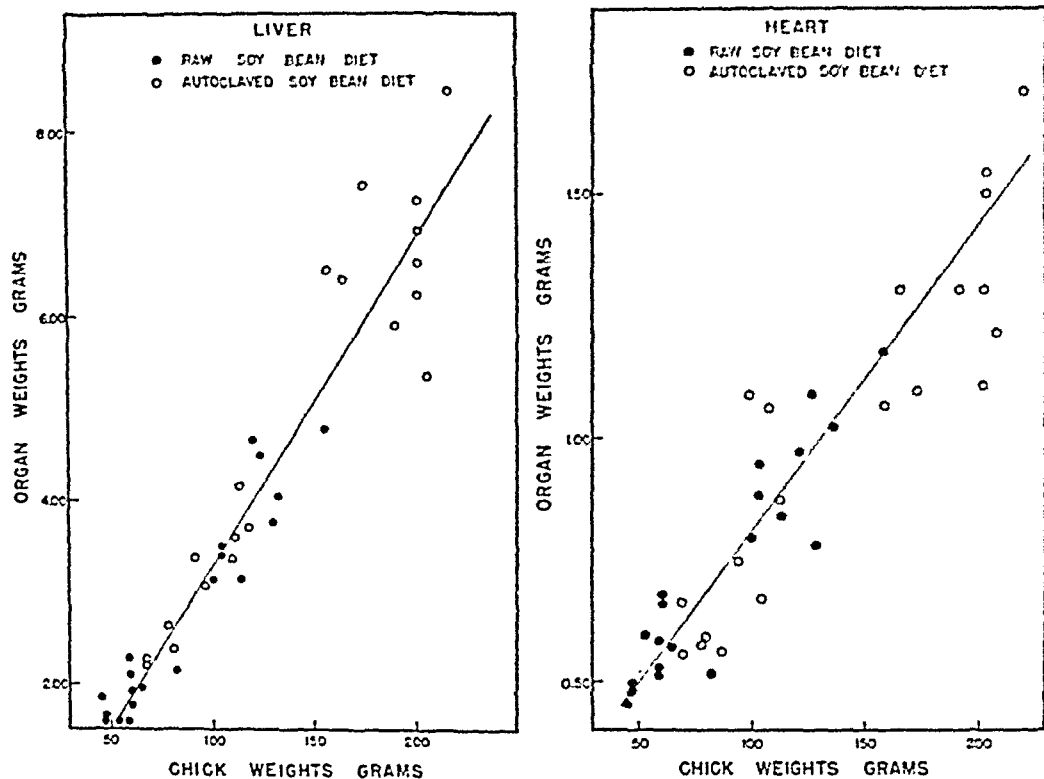


Fig. 2 (left). LIVER WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets. The data were obtained from experiments 2 and 3.

Fig. 3 (right). HEART WEIGHTS OF CHICKS fed raw and autoclaved soy-bean diets. The data were obtained from experiment 3.

The addition of 0.5 per cent methionine to the cooked soy-bean diet influenced neither the size nor the proteolytic activity of the chick pancreas.

#### DISCUSSION

The amount of pancreas in chicks fed a normal diet or a diet containing autoclaved soy beans seldom exceeded 0.5 per cent of their body weights. The values found in birds fed raw soy-bean diets, however, were frequently greater than one per cent. The pancreas responded to the raw soy bean not only in size but in enzyme concentration as well. The concentrations of tryptic activity per gm. of fresh pancreas or per gm. of pancreas nitrogen were greater in chicks fed the raw meal than in those fed the autoclaved meal. This is particularly evident at the higher levels of protein intake, namely 25 to 26 per cent. Supplemental methionine, which at a

high level of protein intake improved the weights of chicks fed the *raw* soy-bean meal, failed to prevent the pancreas hypertrophy or the increased concentration of proteolytic enzymes in this gland.

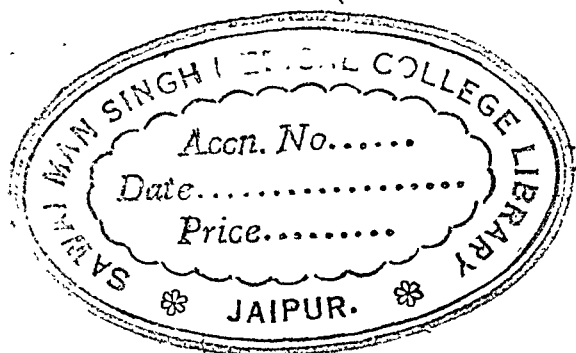
The presence in raw soy-beans of a proteolytic-inhibiting substance was first demonstrated by Bowman (8) and subsequently confirmed by Ham *et al.* (9). Recently Kunitz (2) has isolated from this bean a heat-labile crystalline protein which can combine with an equal weight of crystalline trypsin to form a stable compound having no proteolytic activity. The trypsin-inhibiting action of the soy protein is decreased by proper heat-treatment. It is therefore proposed as a working hypothesis that the increase in size and tryptic content of the pancreas observed here in the birds fed the raw soy bean results from stimulation of the acinar tissue of this gland by either the inhibitor *per se* or by a product of incompletely digested protein. The recent affirmation by Thomas (10) and Ramsay (11) that the presence of peptones in the intestinal tract increases the secretion of the acinar tissue of the pancreas lends support to this hypothesis.

#### SUMMARY

The prolonged feeding of raw soy-bean meal induced in chicks an enlargement of the pancreas and an increase in its proteolytic content. Methionine corrected the growth defect observed in the bird fed a raw soy-bean diet, but failed to prevent the hypertrophy of the pancreas or the increase in its activity. A possible explanation of the pancreas hypertrophy is offered.

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# EFFECT OF PARATHYROID ON RENAL TUBULAR REABSORPTION OF PHOSPHATE AND CALCIUM<sup>1</sup>

IFTAKHAR JAHAN<sup>2</sup> AND ROBERT F. PITTS

*From the Department of Physiology, Syracuse University, College of Medicine*

SYRACUSE, NEW YORK

THE mechanism of action of parathyroid hormone has been the subject of considerable controversy. One of the two main schools of thought holds that it primarily affects the metabolism of phosphorus, the other, that it primarily affects the metabolism of calcium.

Albright (1) has proposed the theory that the hormone, by increasing the urinary loss of phosphate, leads to hypophosphatemia. Because of the reduction in phosphate level, the serum becomes unsaturated with respect to calcium phosphate and, as a consequence, calcium enters the serum from the gastro-intestinal tract and from the bones in increased quantities. Hypercalcemia and hypercalcuria result. Ellsworth (2) maintains that the hyperphosphaturia which follows parathyroid hormone is caused by a lowering of the renal threshold for phosphorus. These investigators as well as others (3-7) have presented clinical as well as experimental evidence in favor of the view that all, or at least a major part, of the action of the hormone is exerted through its effect upon the renal reabsorption of phosphate.

In contrast Selye (8) believes that the hormone acts primarily by stimulating the formation and activity of the osteoclasts of bone, with the result that calcium and phosphorus are mobilized in increased quantities from skeletal stores. Increased mobilization and elevated serum levels account for the increased excretion of both ions. Later Collip *et al.* (9), in a study of the bones of nephrectomized rats that had been injected with parathormone, concluded that the action of the hormone on the bones is independent of any direct influence it may have on the renal threshold for phosphorus.

For a better understanding of the overall action of parathyroid hormone, an exact definition of its effect on the renal tubular reabsorption of phosphate is essential. So far the findings of the various groups that have studied the problem directly have been contradictory. Harrison and Harrison (10) studied reabsorption over a very narrow range of plasma phosphate concentration and concluded that the hormone depresses phosphate reabsorption. Marion Fay and her associates (11) who studied phosphate/creatinine clearance ratios over a wide range of plasma concentration in normal, parathyroid treated, and parathyroidectomized dogs inferred that a lack or an excess of the hormone produced no demonstrable effect upon the capacity of the kidney to excrete, and hence presumably to reabsorb, phosphate.

We felt that the problem merited a thorough investigation; particularly did we feel that the renal tubular reabsorption of inorganic phosphate, in normal and parathyroid treated animals should be studied over a wide range of plasma phosphate concentration and under rigidly controlled conditions of parathyroid activity. The experiments reported below were designed to study this problem.

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Received for publication August 5, 1948.

<sup>1</sup> Aided by a grant from the United States Public Health Service.

<sup>2</sup> Fellow of the Indian Government.

## METHODS

Twelve experiments were performed to assess the effect of parathyroid hormone on the renal tubular reabsorption of inorganic phosphate: 6 with and 6 without the hormone, the latter serving as controls. Eight additional experiments were performed to study the effect of the hormone on calcium reabsorption. All experiments were performed on 2 female mongrel dogs loosely restrained on a comfortable animal board. Preliminary observations were made to find a means of administering the hormone which would ensure a significant increase in serum calcium concentration, stable for the duration of the experiment, and yet exert no gross effect on renal function.<sup>3</sup> The following technique of Collip (12) was found to be most advantageous. Parathyroid extract (Lilly)<sup>4</sup> was given subcutaneously in 2 injections of 3 ml. (U.S.P. 300 U) separated by an interval of 6 hours, starting about 20 hours before the experiment. With this regimen a high and uniform serum-calcium level was obtained at the time the experiment was performed, a level which averaged 13 to 14 mg. per cent, the normal being 8 to 10 mg. per cent. Accordingly the hormone was injected at 12 noon and at 6 P.M. and on the following morning the experiment was performed.

The animals were maintained on a diet of dog biscuits supplemented with meat twice a week. Water, in amounts of 50 ml/kilo body weight, was administered by stomach tube about an hour before the experiment to ensure adequate hydration of the animal. Urines were collected by an indwelling catheter and the bladder was washed with distilled water at the end of each urine collection period. All periods were 10 minutes in length. Two samples of blood, one with and the other without oxalate, were drawn from the jugular vein at the mid-point of each urine collection period. Plasma was used for the analysis of creatinine and phosphate, and serum for calcium. A continuous infusion was administered by the saphenous vein at a rate of 5 ml/min. In the first 12 experiments the infusions contained sodium phosphate (pH 7.4) in quantities sufficient to raise the plasma level in stepwise fashion from a normal value to 1 mM to 5 mM/l., creatinine for the measurement of glomerular filtration rate, and distilled water or 0.9 per cent sodium chloride to render the solution roughly isotonic. In the second group of experiments phosphate was omitted from the infusions.

Creatinine was determined on iron filtrates of plasma and diluted urine by the method of Folin and Wu (13). Phosphate was determined on trichloroacetic acid filtrates of plasma and diluted urine by the method of Fiske and Subbarow (14) as modified by Pitts (15). Calcium was determined on trichloroacetic acid filtrates of serum and evaporated urine by the method of Kramer and Tisdall (16) as modified by Stanford (17), after precipitating the calcium oxalate overnight. All colorimetric analyses were carried out with an Evelyn photoelectric colorimeter.

## RESULTS

The amount of phosphate reabsorbed by the renal tubules in one minute has been calculated as the difference between the quantity filtered through the glomeruli

<sup>3</sup> Large doses of parathormone administered repeatedly reduce glomerular filtration rate and may cause the formation of multiple infarcts in the kidneys.

<sup>4</sup> We are indebted to Eli Lilly and Co. for a generous supply of the hormone.

and the quantity excreted in the urine. The quantity filtered was determined as the product of the glomerular filtration rate in ml/min. and the plasma concentration of phosphate in mM/ml. The quantity excreted was determined as the product of the urine flow in ml/min. and the urinary concentration of phosphate in mM/ml.

The data obtained in 2 typical experiments on one dog, the first a control, and the second following parathormone, are illustrated in table 1. The first 2 horizontal rows of figures in each experiment are data obtained prior to the infusion of phosphate, the remaining 6 rows are data obtained during the infusion of phosphate.

TABLE 1. EXPERIMENTS ILLUSTRATING RELATIONSHIP BETWEEN QUANTITIES OF INORGANIC PHOSPHATE FILTERED THROUGH THE GLOMERULI AND QUANTITIES REABSORBED BY TUBULES AND EXCRETED IN THE URINE IN A NORMAL DOG AND IN THE SAME ANIMAL FOLLOWING ADMINISTRATION OF PARATHORMONE. Dog P.

URINE FLOW	GLOM. FILT. RATE	PHOSPHATE				CALCIUM	
		Plasma	Filtered	Excreted	Reabsorbed	Serum	Excreted
cc/min.	cc/min.	mM/l.	mM/min.	mM/min.	mM/min.	mg.-%	mg/min.
<i>Control</i>							
2.7	52.8	1.05	0.055	0.002	0.053	9.6	0.007
3.45	50.9	1.01	0.051	0.002	0.049	9.6	0.007
3.1	58.6	1.31	0.077	0.011	0.066	9.6	0.052
4.3	59.9	1.51	0.091	0.020	0.071	9.3	0.071
5.7	63.4	2.36	0.150	0.070	0.080	8.7	0.149
5.7	63.9	2.74	0.175	0.093	0.082	8.7	0.161
4.4	62.4	4.55	0.284	0.194	0.090	8.7	0.180
4.25	64.1	5.25	0.336	0.245	0.091	8.0	0.197
<i>With parathormone</i>							
7.56	69.7	0.93	0.065	0.009	0.056	14.1	0.295
8.5	71.5	0.93	0.067	0.008	0.059	14.1	0.348
7.35	73.5	1.40	0.103	0.026	0.077	13.9	0.478
7.35	73.5	1.58	0.116	0.038	0.078	13.6	0.529
4.75	78.2	2.61	0.204	0.096	0.108	13.1	0.551
4.65	76.3	2.80	0.214	0.119	0.095	12.3	0.535
6.8	74.3	4.83	0.357	0.262	0.095	11.9	0.517
6.25	77.7	5.48	0.426	0.323	0.103	11.7	0.531

Plasma phosphate concentration was elevated in stepwise fashion from a normal level of about 1 mM/l. to 5 mM/l. by the infusion of solutions of increasing phosphate content. Although glomerular filtration rate increased progressively throughout the experiment, both this variable and plasma level remained fairly constant in any pair of periods during which a given infusion was administered.

It is evident in the first 2 periods of the 2 experiments, prior to the administration of phosphate, that there was no significant difference either in plasma level or in rate of excretion of phosphate in the normal and in the parathormone treated state. Thus we observe in the control experiment that the plasma phosphate level averaged 1.03 mM/l.; following parathormone it averaged 0.93 mM/l., a value essentially the same as the control. Similarly the rate of excretion of phosphate amounted to 0.002 mM/

min. in the control experiment, a negligibly small value, and remained essentially unchanged at 0.009 mM/min. following parathormone. In sharp contrast, the serum calcium level and the rate of excretion of calcium were considerably elevated by parathormone treatment. Thus in the control experiment the serum calcium level was 9.6 mg. per cent; following the administration of hormone it was elevated to 14.1 mg. per cent. The control rate of calcium excretion was 0.007 mg/min.; following the administration of hormone it rose to 0.348 mg/min. Of most significance, however, is the fact that the normal and the hormone treated animal reabsorbed phosphate at rates which were identical within limits of experimental error over a fairly wide range of plasma phosphate concentration. The maximum reabsorptive capacity (phosphate  $T_m$ ) varied from 0.080 to 0.108 mM/min., a range no greater than that observed under normal conditions. Indeed the higher values were obtained following hormone treatment. It is interesting, although unexplained at the moment, that although the serum calcium level decreased with the infusion of phosphate, the excretion of calcium invariably increased. It is possible that the infusion of phosphate either increased the filterable fraction of serum calcium or in some way interfered with calcium reabsorption.

Figures 1 and 2 summarize the data obtained in 48 clearance comparisons in each of the 2 dogs. The quantity of phosphate reabsorbed by the tubules and excreted in the urine, expressed in mM/min., is plotted against the quantity of phosphate filtered through the glomeruli, likewise expressed in mM/min. It is apparent that the rate of reabsorption of phosphate is essentially the same in the normal and in the parathormone-treated states in both dogs.

Eight additional experiments were performed on the same 2 dogs in a study of the effect of parathormone on the renal tubular reabsorption of calcium. In 4 of the experiments parathormone was administered; in 4 no hormone was given, these latter serving as controls. The method of administration of the hormone and all other experimental procedures were the same as in the experiments just described, except that the infusions contained no phosphate.

In order to calculate the quantity of calcium filtered through the glomeruli it is necessary to determine the fraction of the total serum calcium which is diffusible. To obtain this figure, 10 ml. of serum from each sample of blood were subjected to ultrafiltration under 150 lb. pressure of nitrogen through a cellophane membrane according to the method of Nicholas (18). Consecutive 2 ml. portions of the filtrate as well as the original serum were analyzed for calcium by the methods used in the previous experiments.

Four typical experiments performed on the 2 dogs are illustrated in table 2. For each animal, the first 3 rows of figures constitute control observations; the last 3 rows constitute observations following the administration of parathormone. It is evident in these experiments as in those described above that there is no significant difference in serum phosphate level, in rate of reabsorption or in rate of excretion of phosphate in the control and in the parathyroid treated state. All of our observations have been completely consistent in this respect. However, we must point out that our experiments have all been performed from 20 to 23 hours after the initial dose and from 14 to 17 hours after the final dose of parathormone. Thus any

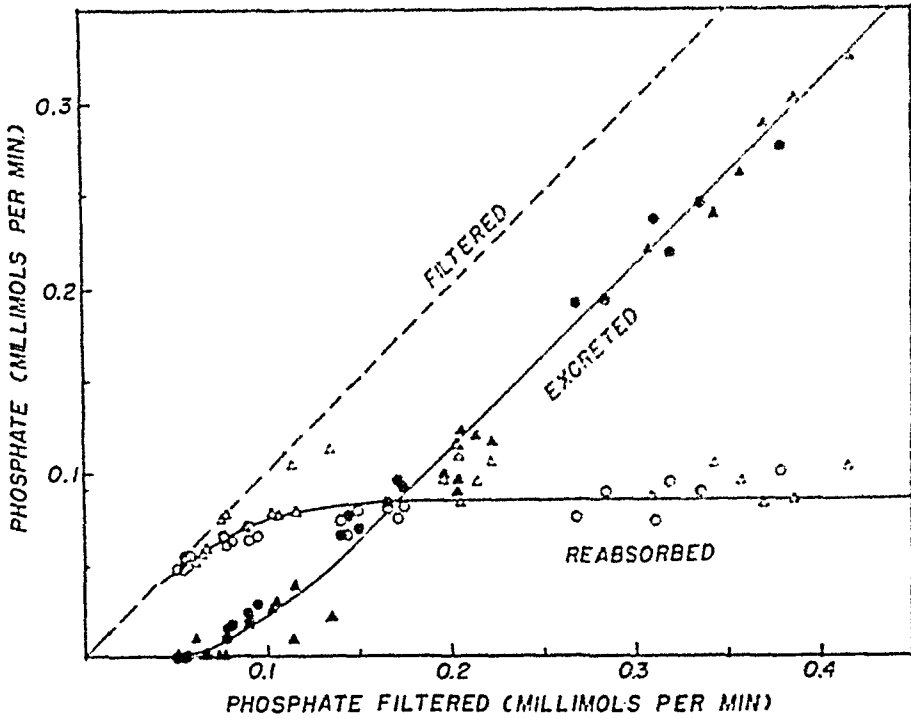


Fig. 1. RELATIONSHIP between quantity of inorganic phosphate filtered through glomeruli and quantities reabsorbed by renal tubules and excreted in the urine. *Dog P.* Circles, control observations; triangles, following parathormone.

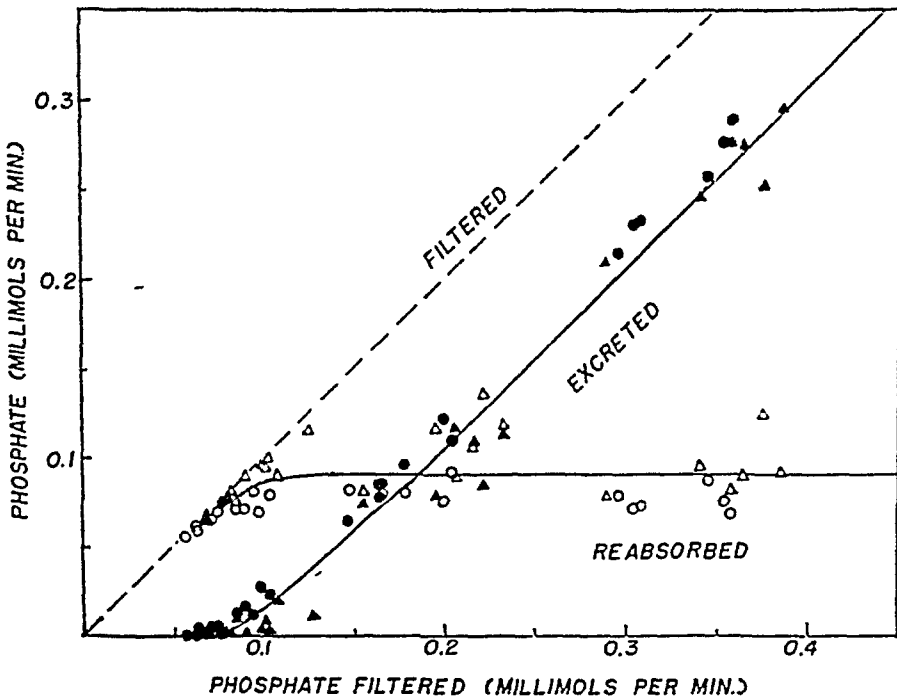


Fig. 2. RELATIONSHIP between the quantity of inorganic phosphate filtered through the glomeruli and the quantities reabsorbed by the renal tubules and excreted in the urine. *Dog R.* Circles, control observations; triangles, following parathormone.

transitory effect of the hormone on phosphate excretion or reabsorption would be missed.

In contrast it is evident from table 2 that the serum concentration of calcium and the rates of reabsorption and excretion of calcium were all significantly increased by parathormone administration. Thus during the control periods the serum concentration of total calcium averaged 6.9 mg. per cent in *dog R* and 7.7 mg. per cent in *dog P*;<sup>5</sup> that of filterable calcium averaged 4.1 mg. per cent in *dog R* and 4.7 mg. per cent in *dog P*. Following parathyroid hormone, serum total calcium increased

TABLE 2. EXPERIMENTS ILLUSTRATING RELATIONSHIP BETWEEN QUANTITIES OF CALCIUM FILTERED THROUGH GLOMERULI AND QUANTITIES REABSORBED BY TUBULES AND EXCRETED IN URINE IN A NORMAL DOG AND IN THE SAME ANIMAL FOLLOWING ADMINISTRATION OF PARATHORMONE.

GLOM. /FILT. RATE	URINE FLOW	SERUM			PHOSPHATE			CALCIUM		
		Phos- phate	Total calcium	Filtrate calcium	Filtered	Excreted	Re- absorbed	Filtered	Excreted	Re- absorbed
cc/min.	cc/min.	mM/L.	mg%.	mg%.	mM/min.	mM/min.	mM/min.	mg/min.	mg/min.	mg/min.
<i>Dog R; Control</i>										
69.2	5.9	0.79	7.0	4.2	0.055	0.0005	0.055	2.91	0.004	2.90
70.2	7.1	0.71	6.8	4.1	0.050	0.0001	0.050	2.88	0.011	2.87
69.2	7.05	0.68	6.8	4.1	0.047	0.0001	0.047	2.84	0.029	2.81
<i>Dog R; Parathormone</i>										
71.7	6.5	0.88	11.1	5.8	0.063	0.0009	0.062	4.16	0.150	4.01
66.5	4.9	0.85	11.0	6.8	0.057	0.0001	0.057	4.52	0.220	4.30
71.2	2.1	0.84	11.5	7.0	0.060	0.0001	0.060	4.99	0.350	4.64
<i>Dog P; Control</i>										
60.9	3.05	0.64	7.0	4.6	0.039	0.0001	0.039	2.80	0.0009	2.80
63.6	1.65	0.63	7.8	4.8	0.040	0.0002	0.040	3.05	0.002	3.05
66.6	1.15	0.61	8.3	4.6	0.041	0.0002	0.041	3.06	0.004	3.06
<i>Dog P; Parathormone</i>										
62.6	5.7	0.90	12.7	6.9	0.056	0.0002	0.056	4.32	0.250	4.07
60.9	3.25	0.90	12.3	6.5	0.055	0.0001	0.055	3.96	0.400	3.56
62.0	1.7	0.78	12.8	6.7	0.048	0.0002	0.048	4.15	0.320	3.83

to 11.2 and 12.6 mg. per cent, and filterable calcium to 6.5 and 6.7 mg. per cent respectively in the 2 animals.

It is apparent from the columns labelled calcium filtered, excreted and reabsorbed, that the increased delivery of calcium into the renal tubules, in consequence of the increase in serum concentration of filterable calcium, adequately accounts for the calcuria observed. Thus in *dog R* the quantity filtered rose from 2.88 to 4.56 mg/min., and in *dog P* from 2.97 to 4.14 mg/min. In *dog R* the quantity excreted rose from 0.015 to 0.240 mg/min., and in *dog P* from 0.002 to 0.323 mg/min. Since the increase in the quantity filtered greatly exceeded the increase in the quantity excreted, it is apparent that the quantity reabsorbed likewise increased, from 2.86 to

<sup>5</sup> The cause of the low initial calcium in both of the control experiments is unknown. However the administration of parathormone in each instance increased serum calcium nearly 100%.



4.33 mg/min. in *dog R*, and from 2.90 to 3.82 mg/min. in *dog P*.<sup>6</sup> We feel that this increase in reabsorption is the consequence of the presentation of increased quantities of calcium to tubules whose reabsorptive capacities are unsaturated at ordinary plasma levels.

#### DISCUSSION

The experimental results presented above demonstrate that the administration of 600 U of parathormone causes no appreciable depression of the level of phosphate in the serum and no significant interference with the capacity of the renal tubules to reabsorb phosphate some 14 hours later. Nevertheless at this time the serum concentration of calcium is maximally increased and calcium is excreted in the urine in large quantities. Because the hypercalcemia and hypercalcuria persist for many hours, it is apparent that they cannot be causally related to any transient phosphaturia which immediately follows the administration of the hormone, but which subsides before the serum level of calcium and rate of excretion of calcium reach their maxima. Our experiments are therefore consistent with the conclusion of Collip *et al.* (9) that the action of parathormone on the metabolism of calcium is independent of any direct influence it may have on the renal threshold for phosphorus. Indeed we would go further in saying that at the time of greatest mobilization and urinary loss of calcium, the hormone exerts no effect on the reabsorption or excretion of phosphate by the kidney.

It is likewise evident that parathormone does not interfere with calcium reabsorption by the renal tubules. Indeed there is greater reabsorption of calcium in the hyperparathyroid state than under normal conditions. This increased reabsorption of calcium must mainly result from the fact that the kidney tubules are presented with greatly increased quantities of calcium in the glomerular filtrate. Although more of the filtered calcium is reabsorbed, more also spills over into the urine to account for the evident hypercalcuria.

Monahan and Freeman (19) similarly believe that the parathyroid gland exerts an influence on calcium metabolism that is independent of the kidneys. Thus they observed a decrease in the serum calcium concentration amounting to about 50 per cent in 72 hours in nephrectomized parathyroidectomized dogs, whereas no similar decrease was observed in nephrectomized controls. The serum inorganic phosphate was markedly elevated in both groups. Recently Ingalls, Donaldson and Albright (20) produced the characteristic bone lesions of hyperparathyroidism by the administration of parathormone to nephrectomized rats. They are also of the opinion that their evidence supports the view that parathormone has a direct action on bone.

It is concluded from these and from our own studies that hypercalcuria and hypercalcemia are dependent on extrarenal actions of the hormone and not on any specific effect on the renal tubular reabsorption of either phosphorus or calcium.

#### SUMMARY

The effect of parathormone on the renal tubular reabsorption of inorganic phosphate has been studied over a range of plasma concentration of 0.9 to 5.48 mM/liter.

<sup>6</sup> Comparable results were obtained in 4 additional experiments.

Under the conditions of our experiments, the rate of reabsorption of phosphate is essentially the same in the parathormone-treated and in the normal animal. The rate of reabsorption of calcium is greater following parathormone, for in consequence of increased plasma level of filterable calcium, increased quantities of calcium are presented to the tubules in the glomerular filtrate. Hypercalcemia and hypercalcuria produced by the administration of parathormone are dependent on extrarenal actions of the hormone in mobilizing calcium from body stores, not on any specific depression of the renal tubular reabsorption of either phosphorus or calcium.

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# EFFECT OF ETHER AND PENTOBARBITAL ANESTHESIA ON CERVICAL LYMPH FLOW AND PROTEIN CONTENT IN THE CAT<sup>1</sup>

MARIE-LOUISE FLINKER<sup>2</sup> AND JANE D. MCCARRELL<sup>3</sup>

*From the Department of Physiology, Vassar College*

POUGHKEEPSIE, NEW YORK

THE volume of lymph flow and protein concentration of lymph in the dog have been shown by Polderman *et al.* (1) to be markedly affected by the type of anesthesia. Pentobarbital sodium caused a reduction in flow of about 50 per cent and ether caused an increase of about 50 per cent over the flow under local procaine anesthesia. It was the purpose of the present problem to study the effects of ether and pentobarbital sodium anesthesia on lymph flow and protein concentration of lymph in the cat and to compare these results with the effects noted in the dog by other workers.

## METHOD

Fifteen cats weighing 2.4 to 4.4 kg. were used. These experiments were divided into four groups according to the anesthetic used or the order in which the anesthetics were administered. The first group consisted of the determination of the flow of lymph when the animal was first anesthetized with pentobarbital sodium and then, when the flow had reached a relatively steady state, with ether. The second group consisted of animals first anesthetized with ether followed by pentobarbital sodium when the flow had leveled off. The other two groups were control experiments and consisted of the determination of the effects of ether alone and of pentobarbital sodium alone. The animals were kept at a relatively light level of surgical anesthesia. The corneal reflex was used as an indicator of degree of anesthetization, except in a few isolated cases where it was absent. The respiratory rate then had to be relied upon to indicate the relative depth of anesthesia.

A 4 per cent solution of pentobarbital sodium was used. An initial dose of 40 mg/kg. was given intraperitoneally, and all subsequent doses needed to keep the level of anesthesia constant were given intravenously. A tracheal cannula was inserted and a small bottle partially filled with water was attached to the cannula. In this way the same amount of dead space was present under pentobarbital sodium as under ether.

When the cat was anesthetized with ether first, it was placed in a specially adapted can so constructed that ether vapor could be introduced. There was a small observation window in the top of the can. When the cat was anesthetized, it was transferred to the operating board and ether was administered through a cone using the drop method until a tracheal cannula could be inserted. A small bottle, through which the flow of ether vapor and air could be adjusted, was partially filled with ether and attached to the tracheal cannula.

Physiological saline (20 cc/kg.) was slowly administered to each cat intravenously through a

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Received for publication May 3, 1948.

<sup>1</sup> Submitted in partial fulfillment of requirements for the Degree of Master of Arts in Vassar College.

<sup>2</sup> Present address: Department of Physiology and Pharmacology, Medical College of Virginia, Richmond, Va.

<sup>3</sup> Present address: Department of Biology, Hood College, Frederick, Md.

femoral vein before lymph collection was begun, to insure adequate tissue hydration. The mean arterial pressure was recorded directly from a femoral artery using a mercury manometer. Blood samples were taken at regular intervals from the other femoral artery which had also been cannulated. Blood collected in capillary tubes was centrifuged and the hematocrit values determined from these samples. Serum protein determinations were done using a Zeiss dipping refractometer. Body temperatures, respiration rate and depth were noted.

The right and left cervical lymphatic ducts were isolated in all but a few cases when one or the other could not be found. Both lymphatics were cannulated in some cases. When only one lymphatic was cannulated, the other was tied off. A thin wire, dipped in dry heparin, was inserted in each cannula to prevent coagulation. In order to insure a continuous flow of lymph, a 'nodding preparation' was used (2).

The lymphatic cannulae were emptied at 5-minute intervals using a 'mosquito bill' pipette. All the lymph for a 15-minute period was collected in a single, previously weighed test tube. The protein concentration of the lymph was determined using a Zeiss dipping refractometer.

### RESULTS

A summary of the data obtained is presented in tables 1 and 2. The values listed represent the average of the results secured during the period of one hour. The hour periods chosen were those during which the lymph flow had more or less leveled off. Whenever possible, two consecutive hours were used to note any immediate effects of changing the anesthetic agent. Figures 1-4 are graphs showing the course of four typical experiments, each one representing one type of experiment.

An examination of the average results reveals no marked changes in lymph flow when pentobarbital sodium anesthesia was followed by ether or when the reverse was the case. There was some variation in individual experiments, but there was a trend toward decreasing flow as the experiment progressed, regardless of the anesthetic employed. A slight rise in lymph protein content was found when pentobarbital sodium followed ether anesthesia, but no consistent change was noted when ether was introduced after pentobarbital sodium. The changes which occurred were of approximately the same magnitude as those found in the control experiments when ether or pentobarbital sodium alone was used for the duration of the experiment.

The hematocrit values decreased slightly when ether anesthesia was followed by pentobarbital sodium. This was also true of the serum protein values. On the other hand, when ether was introduced after pentobarbital sodium anesthesia, the effect was inconsistent; a rise in hematocrit occurred in two cases and a drop of the same magnitude occurred in the third. A majority of the serum protein values increased. The mean arterial pressure remained fairly constant in all experiments and the respiration rate showed no excessive variation.

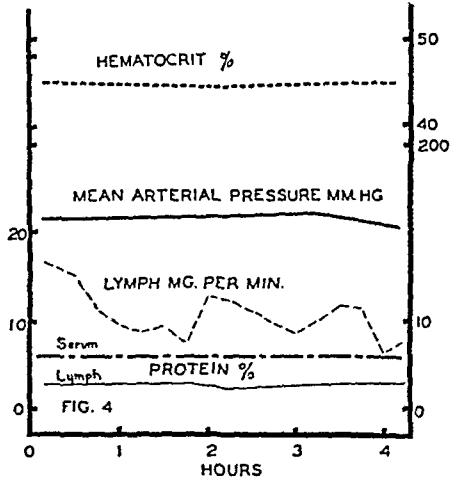
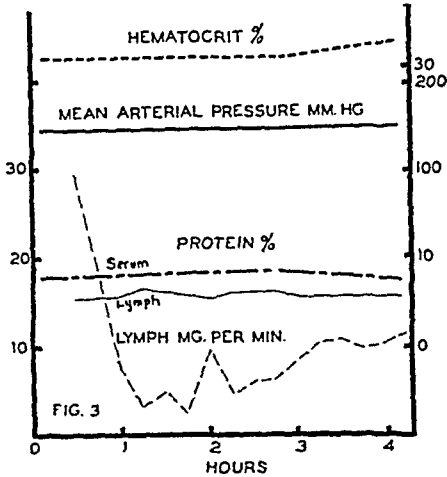
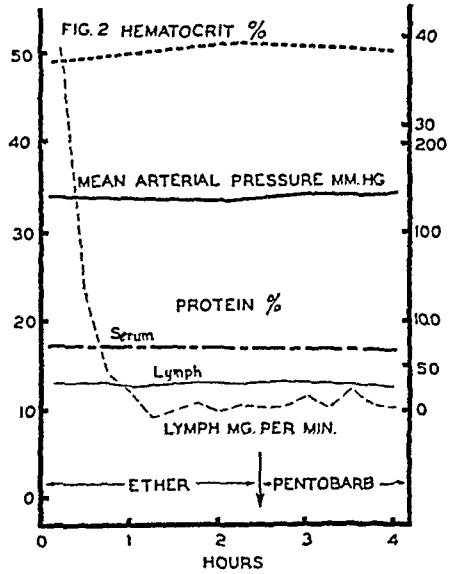
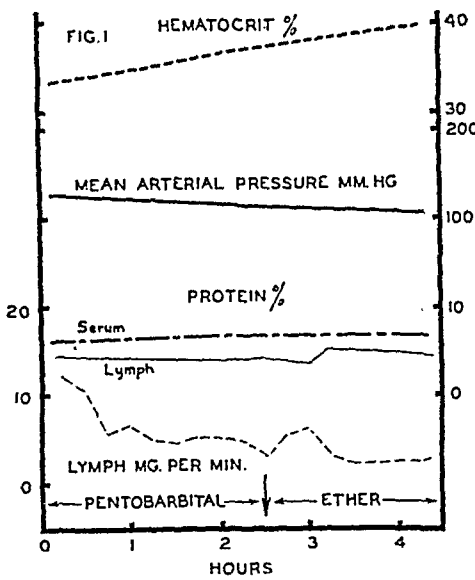
The graphs show that, regardless of the anesthetic agent used at the outset of the experiment, there was a large initial flow of lymph. This was due to an accumulation of tissue fluid and lymph in the region of the head and neck during the period which preceded the beginning of the nodding motion. When the head movement was begun, the accumulated fluid was passively pushed into the cervical lymphatic ducts and out into the cannulae in relatively large amounts. However, in most cases the flow had dropped perceptibly by the end of an hour and had begun to level off during the second hour. Since the values obtained during the first hour are not indicative of the effect of the anesthetic used, they will be disregarded.

A certain amount of irregularity in lymph flow was encountered in all experiments. These variations cannot be accounted for by attributing them to changing the anesthetic used, since they occurred in control experiments as well.

TABLE 1. A. PENTOBARBITAL FOLLOWED BY ETHER ANESTHESIA. B. ETHER FOLLOWED BY PENTOBARBITAL ANESTHESIA

NO.	WT.	LYMPH		BLOOD			RESPIRATIONS/MIN.
		Flow	Protein	Hematocrit	Protein	Mean arterial pressure	
	kg.	mg/min.	%	%	%	mm. Hg	
<i>A. Pentobarbital Anesthesia</i>							
1 ♀	3.0	4.9	3.71		6.27	110	24
2 ♀	3.5	17.7	3.54		5.65	148	26
3 ♀	2.6	10.0	3.10		4.98	144	25
4 ♀	2.4	13.3	3.01		5.46	116	26
5 ♂	3.9	5.95	3.45	32.33	6.73	114	27
6 ♂	3.7	7.7	4.45	44.67	7.03	188	28
7 ♂	2.7	4.95	4.07	37.11	6.63	114	40
Average.....		9.2	3.62	38.04	6.11	133	28
<i>A. Ether Anesthesia</i>							
1		5.5	3.41		6.70	120	29
2		7.6	3.89		5.92	152	32
3		6.6	3.48		5.84	160	27
4		11.4	3.31		5.49	116	21
5		11.1	3.11	35.46	6.78	96	26
6		10.9	3.80	42.34	7.13	188	30
7		4.4	4.22	40.00	6.58	104	46
Average.....		8.2	3.60	39.27	6.35	134	30
<i>B. Ether Anesthesia</i>							
8 ♂	4.1	5.5	3.75	49.00	8.40	120	20
9 ♂	3.8	10.4	3.05	39.10	7.06	136	29
10 ♀	3.0	8.3	2.48	45.16	5.97	140	31
11 ♀	3.9	15.7	3.05	44.45	6.47	108	68
Average.....		9.97	3.08	44.43	6.98	126	37
<i>B. Pentobarbital Anesthesia</i>							
8		6.1	4.09	48.9	8.07	111	21
9		10.9	3.08	38.2	6.78	140	27
10		10.4	2.66	40.34	5.93	136	40
11		10.4	3.69	38.72	6.38	106	64
Average.....		9.5	3.38	41.54	6.79	123	38

The data obtained indicate that ether and pentobarbital sodium have no significant effect on lymph flow and protein concentration in the cat.



CHARTS ILLUSTRATING TYPICAL EXPERIMENTS. Arrows indicate the times at which anesthesia was changed.

Fig. 1. Pentobarbital sodium followed by ether anesthesia. This is one of the experiments in which the hematocrit increased. (Cat 7)

Fig. 2. Ether anesthesia followed by pentobarbital. (Cat 9)

Fig. 3. Pentobarbital sodium anesthesia only (Cat 13). Values for lymph protein and lymph were obtained by combining the yield from the right and left cervical lymphatic ducts.

Fig. 4. Ether anesthesia only (Cat 15).

#### DISCUSSION

In the light of the marked changes in lymph flow and protein content obtained by Polderman, McCarrell and Beecher (1) using ether and pentobarbital sodium anesthesia in the dog, the changes observed during these experiments on the cat cannot be called significant. No great increase in lymph flow under ether anesthesia or large decrease with pentobarbital sodium was noted. There is therefore apparently a species difference with regard to the effects of these two anesthetics on the lymph flow in the cat and dog. It has long been known that various anesthetic agents have different effects on the blood. It has been shown that ether causes hemoconcentration in dogs (3-10). Pentobarbital sodium causes hemodilution in

dogs (7, 8, 10, 11, 12). There are indications that ether anesthesia decreases plasma volume in the human being (13, 14).

Hamlin and Gregersen (15) presented data which indicated that intravenous injection of nembutal caused an immediate increase in plasma volume of cats. Jarcho (10) found in cats that although nembutal anesthesia caused a marked decrease in hematocrit and plasma protein concentration, subsequent anesthetization of the cats with ether did not produce changes in blood concentration. Conley (16) using ether, reported finding no change in plasma volume of cats. If such a species difference can be shown to exist with regard to the effect of one anesthetic on the concentration of blood in these animals, it is conceivable that this difference will be reflected in the lymph flow.

TABLE 2. CONTROL EXPERIMENTS

NO.	WT.	LYMPH		BLOOD			RESPIRA- TIONS/MIN.
		Flow	Protein	Hematocrit	Protein	Mean arte- rial pressure	
	kg.	mg/min.	%	%	%	mm. Hg	
<i>Pentobarbital Anesthesia only</i>							
12♂	3.5	13.4	5.49		7.44	122	36
13♀	3.7	9.3 <sup>1</sup>	5.73 <sup>1</sup>	31.76	7.84	148	15
Average.....		11.4	5.61	31.76	7.64	135	26
<i>Ether Anesthesia only</i>							
14♀	2.8	10.1	2.76	44.59	5.98	114	41
15♂	4.4	29.3	5.35	47.87	8.36	135	21
Average.....		19.7	4.06	46.23	7.17	125	31

<sup>1</sup> Combined flow from right and left cervical lymphatics.

## SUMMARY

Ether anesthesia in comparison with pentobarbital sodium anesthesia had no significant effect on lymph flow and lymph protein concentration in the cat, thus suggesting a species difference between the cat and dog with respect to these anesthetics.

## ADDENDUM

Since these experiments were performed, Smith *et al.* (17) have reported results obtained with regard to the immediate effects of ether and nembutal upon some blood components in the cat which indicate the immediate effect of ether on the blood is hemoconcentration and of nembutal, hemodilution.

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# CHOLINESTERASE ACTIVITY IN NORMAL AND FEBRILE RABBIT BRAIN<sup>1</sup>

C. N. PEISS, JOHN FIELD AND VICTOR E. HALL

*From the Department of Physiology, Stanford University School of Medicine*

STANFORD UNIVERSITY, CALIFORNIA

IT IS generally assumed that in fever the temperature regulating center is set at a supranormal level and that active regulation occurs at this new level (1). It seems possible that this change in setting involves a metabolic change in the temperature regulating center. This change might involve an alteration in intracellular enzyme pattern which could be detected by enzyme assay. Technical difficulties precluded direct metabolic investigation of the cells of the hypothalamic centers. However, because of the qualitative similarity of the metabolic pattern in various parts of the central nervous system (2, 3), quantitative changes appearing in one region might be expected to be paralleled by changes in a similar direction in other regions. In view of current interest in the possibility that the acetylcholine-cholinesterase system is a factor in nerve function (4-6), we have compared the cholinesterase activity of homogenates prepared from brain tissue of normal and febrile rabbits. The results are reported in this paper.

Cholinesterase catalyses the hydrolysis of acetylcholine, with the formation of choline and acetic acid. The chemical and physiological properties of acetylcholine and the enzymes concerned with its synthesis and degradation have been reviewed by various workers (4-7). The manometric determination of cholinesterase is based on the displacement of CO<sub>2</sub> from a bicarbonate buffer by the acetic acid formed in the reaction. Thus hydrolysis of 1 mol of acetylcholine yields 1 mol of acetic acid, which in turn displaces 1 mol of CO<sub>2</sub> from the buffer medium (8-10).

## METHODS

Cholinesterase was measured manometrically with the conventional Warburg technique. The suspension medium was Ringer's-bicarbonate buffer (calcium-free). Merck acetylcholine chloride was used as substrate in a final vessel concentration of 0.015 M and was made up in the suspension medium. The buffer solution was gassed for 20 minutes with a mixture containing 95 per cent N<sub>2</sub>-5 per cent CO<sub>2</sub> before the vessels were filled. After the vessels had been filled with all necessary solutions, they were placed in a water bath which was maintained at a temperature of  $39 \pm 0.01^\circ\text{C}$ . They were then gassed for 10 minutes with the 95 per cent N<sub>2</sub>-5 per cent CO<sub>2</sub> mixture. After a 10-minute equilibration period, the acetylcholine solution was added from the sidearm of the vessels and readings were begun. Readings were made every 5 minutes for a 40-minute period. The rate of evolution of CO<sub>2</sub> during this time was constant.

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Received for publication July 19, 1948.

<sup>1</sup> The work reported here was done under a contract between the Air Materiel Command, Wright Field, Dayton, Ohio and Stanford University.

Cholinesterase activity was determined for whole homogenate from rabbit brain and for the supernatant fraction of the homogenate. It has been shown previously that the supernatant fraction of the homogenates prepared in the Waring Blendor exhibits 35 to 45 per cent of the total activity of the whole homogenate (11), whereas a corresponding value of 10 to 20 per cent (11) is obtained when the Potter-Elvehjem homogenizer is used (12). Accordingly, all of our homogenates were prepared with the Waring Blendor. In order to maintain constant conditions throughout the experiments, all homogenates contained about 200 mg. fresh tissue per ml. Homogenates were prepared in Ringer's-bicarbonate buffer. A typical reaction flask mixture is given below:

Acetylcholine chloride, 0.15 M in Ringer's-bicarbonate buffer.....	0.15 ml.
Ringer's-bicarbonate buffer.....	1.10 ml.
Homogenate or supernatant.....	0.25 ml.

Two rabbit brains were used for each experiment reported here. Of these, one served as a control and is hereafter referred to as the 'normal'. The other rabbit received an injection of 0.05 ml. typhoid-paratyphoid triple vaccine<sup>2</sup> intravenously, and is hereafter referred to as the 'febrile'. Rectal temperature was checked from the time of injection throughout a period of one hour. A rise in rectal temperature of 1.0°C. was considered a satisfactory response. In most cases, the rise in rectal temperature amounted to 1.2 to 1.5°C. Exactly one hour after the time of vaccine injection, the 2 animals were killed by injection of air into the marginal ear vein. The brains were rapidly excised and placed in beakers surrounded by cracked ice. The homogenates were then prepared immediately in the cold room at 0.0°C.

#### RESULTS AND DISCUSSION

It is shown in table 1A that the values for cholinesterase activity in normal and in febrile rabbit brain homogenates are very similar. The difference in the mean values has been analysed statistically and has been shown to be insignificant ( $P = 0.26$ ). Values are expressed both in terms of CO<sub>2</sub> production and of milligrams of acetylcholine hydrolyzed for convenience in comparison with other data in the literature. These values are calculated on the basis of 100 mg. fresh tissue per hour. Similar results for the supernatant fraction are shown in table 1B. Here, the agreement between normal and febrile animals is even more striking. It is readily apparent from inspection of the data that there is no significant difference between the two sets of values ( $P = 0.81$ ). The results in table 1B are based on 1.0 ml. of a supernatant from a homogenate containing 100 mg. fresh tissue per ml. In this way it is possible to correlate the relative activity of the whole homogenate and of the supernatant fraction. It is interesting to note that the activity of the supernatant fraction amounts to about 40 per cent of that of the whole homogenate, which confirms the choice of the Waring Blendor when a high activity is desired in the supernatant.

<sup>2</sup> Cutter Laboratories Typhoid-Paratyphoid Vaccine, containing 1000 million *E. typhosa* organisms per cc., 500 million *S. paratyphi* organisms per cc. and 500 million *S. schottmuelleri* organisms per cc.

The results presented here are in close agreement with those reported by other workers for normal rabbit brain cholinesterase activity. Nachmansohn and Feld (11) report that normal rabbit brain homogenate hydrolyzes 8.0 mg. acetylcholine per 100 mg. fresh tissue per hour. DuBois and Magnum (10) have found that normal rabbit brain homogenate produces 1224 microliters CO<sub>2</sub> per 100 mg. fresh tissue per hour, which is equivalent to the hydrolysis of 9.92 mg. acetylcholine per 100 mg. fresh tissue per hour. Our results of 9.07 mg. acetylcholine hydrolysed per 100 mg. fresh tissue per hour fall midway between these values. The results presented here represent, of course, the sum of cholinesterase activity in the various parts of the brain. Activity varies widely in different areas, as shown for rabbit

TABLE 1

	NORMAL		FEBRILE	
	μl. CO <sub>2</sub> evolved	mg. ACh hydrolyzed	μl. CO <sub>2</sub> evolved	mg. ACh hydrolyzed
PART 1A. Cholinesterase activity of whole homogenate from normal and febrile rabbit brain. (Values/100 mg. fresh tissue/hour.) The symbols S.D. and N stand for the standard deviation and the number of runs respectively				
Mean.....	1119	9.07	1087	8.81
Range.....	982-1242	7.95-10.06	1039-1209	8.43-9.81
S.D.....	79.58	0.645	49.40	0.403
N.....	12	12	12	12
PART 1B. Cholinesterase activity of supernatant fraction of homogenates from normal and febrile rabbit brain. (Values per 1.0 ml. supernatant from a 100 mg/ml. homogenate/hour.)				
Mean.....	435	3.52	433	3.51
Range.....	417-467	3.38-3.78	420-453	3.40-3.67
S.D.....	14.77	0.118	12.37	0.101
N.....	12	12	12	12

brain by Nachmansohn and Feld (11). For example, whole homogenate from the cerebral cortex of normal rabbit brain hydrolyzes 4.5 mg. acetylcholine per 100 mg. fresh tissue per hour, while a homogenate prepared from the nucleus caudatus is able to hydrolyze 31.6 mg. acetylcholine per 100 mg. fresh tissue per hour.

SUMMARY

Whole homogenate from normal and febrile rabbit brains hydrolyzes respectively 9.07 and 8.81 mg. of acetylcholine per 100 mg. fresh tissue per hour. These values are the means of twelve determinations. The difference between these means is not significant. The supernatant fraction of similar homogenates hydrolyzes respectively 3.52 and 3.51 mg. acetylcholine per 100 mg. fresh tissue per hour (mean of 12 determinations each). These means are also not significantly different. To the extent that these assays on whole brain can cast light upon processes mediating temperature regulation, they suggest that quantitative changes in cholinesterase assay are not associated with the 'thermostatic reset' which occurs in fever.

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# DISTRIBUTION OF THE TWO FRACTIONS OF CHOLINESTERASE IN HOMOGENATES OF PORTIONS OF THE DOG BRAIN<sup>1</sup>

J. MAXWELL LITTLE

*From the Department of Physiology and Pharmacology, The Bowman Gray School of Medicine of Wake Forest College*

WINSTON-SALEM, NORTH CAROLINA

IT HAS been reported previously (1) that two fractions of specific cholinesterase are present in homogenates of mouse brain. One is found in the precipitate obtained by centrifuging the homogenate, and one remains in the supernatant fluid. The precipitate fraction is considerably more heat labile than is the supernatant fraction.

The present report is concerned with the total cholinesterase activity of homogenates of portions of the dog brain and the distribution of the two above fractions in the homogenates. In addition, the heat lability of the two fractions is reported.

## PROCEDURE

Dogs were killed with pentobarbital sodium (*dog 1*) or with ether and chloroform (*dogs 2 and 3*). The brain and a portion of the cervical spinal cord were removed as quickly as possible. The brain of *dog 1* was dissected<sup>2</sup> immediately and the various portions were stored in closed containers in a CO<sub>2</sub> ice box until used. The brains of *dogs 2 and 3* were stored immediately in the CO<sub>2</sub> ice box. They were subsequently dissected, without thawing, and the various portions were returned to the ice box until used.

The tissue was homogenized, after thawing at room temperature in a fluid with the following composition: NaCl 0.15 M, MgCl<sub>2</sub> 0.04 M, and NaHCO<sub>3</sub> 0.025 M. The final substrate concentration was 0.015 M acetylcholine. The remainder of the procedure followed was that previously reported (1).

## RESULTS

It will be seen in table 1 that when the supernatant fluid and precipitate fractions were heated at 53°C. for 60 minutes the average remaining cholinesterase activity of the supernatant fractions was 42.2 per cent of that present before heating, while the average remaining activity of the precipitate fractions was only 29.4 per cent of that present before heating. There was less remaining activity in each individual precipitate fraction than in the supernatant fluid fraction with the exception of the cerebellar peduncle and the internal capsule. These data are interpreted as evidence that the precipitate fraction of most portions of the dog brain is more heat labile than is the supernatant fraction.

In table 2 the cholinesterase activity of homogenates of the various portions of the brain which were studied will be found. It will be seen that the enzyme activity

Received for publication August 18, 1948.

<sup>1</sup> Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

<sup>2</sup> The author is indebted to Dr. H. D. Green for the dissections of the brains.

varied considerably from one portion of the brain to another, the lowest being no detectable activity in the corpus callosum and the greatest activity being found in the caudate nucleus. It will be seen also that there is considerable variability among the various brain tissues in the per cent of the total activity which remains

TABLE 1. EFFECT OF HEATING UPON THE CHOLINESTERASE ACTIVITY OF SUPERNATANT FLUIDS AND PRECIPITATES OBTAINED FROM HOMOGENATES OF PORTIONS OF DOG BRAIN  
(QA·Ch = mg. of acetylcholine hydrolyzed/100 mg. wet weight/60 min.)

TISSUE	CONTROL QA·Ch.		PERCENTAGE OF CONTROL ACTIVITY REMAINING AFTER 60 MIN. HEATING AT 53°C.	
	Supernatant	Precipitate	Supernatant	Precipitate
Cerebellar nuclei . . . . .	2.52	6.13	38	21
Cerebellar peduncle . . . . .	3.15	3.73	29	29
Cerebellar cortex . . . . .	3.08	8.91	55	13
Cerebellar cortex . . . . .	2.30	4.12	31	21
White matter . . . . .	0.44	0.56	70	54
Caudate nucleus . . . . .	8.59	34.99	22	12
Caudate nucleus . . . . .	6.46	16.82	33	21
Caudate nucleus <sup>1</sup> . . . . .	7.92	17.77	20	10
Internal capsule . . . . .	1.15	1.70	60	60
Medulla . . . . .	1.76	3.27	57	36
Thalamus . . . . .	1.68	3.21	49	46
Average . . . . .			42.2	29.4

<sup>1</sup> Heated at 54°C.

in the supernatant fluid fraction when the total homogenate is centrifuged. The supernatant fluid enzyme activity varies between an average of 27 per cent for the cerebellar cortex to a value of 55 per cent for the rostral portion of the cervical spinal cord.

#### DISCUSSION

With the exception of the caudate nucleus, the values reported here for the activity of the uncentrifuged homogenate agree generally with those reported by Nachmansohn (2). The values for the caudate nucleus are somewhat lower than those reported by Nachmansohn.

On the basis of the data presented in table 1, it appears that two fractions of cholinesterase are present in dog brain homogenate. The distribution of the two fractions between the supernatant fluid and the precipitate, obtained by centrifuging the homogenate, is considerably different from that reported for mouse brain (1) where the average values for supernatant fluid and precipitate were respectively 16.8 per cent and 82.9 per cent.

It will be seen in table 2 that as the estimated relative mass of nerve fibers in the tissues examined increases the general trend is for an increase in the per cent of the total cholinesterase activity which is found in the supernatant fluid from the homogenate. An exception to this is the corpus callosum, which had an enzyme

activity so low that it was not detectable with the procedure used here. It is possible that the same relationship would be found in this tissue if a more sensitive procedure were used. The relationship between the nerve fiber mass and the activity of the

TABLE 2. CHOLINESTERASE ACTIVITY OF HOMOGENATES OF PORTIONS OF DOG BRAIN AND DISTRIBUTION OF ACTIVITY BETWEEN SUPERNATANT FLUID AND PRECIPITATE OBTAINED FROM THESE HOMOGENATES

DOG NO.	TISSUE	ESTIMATED % NERVE FIBERS	TOTAL $Q_{A \cdot Ch}$	SUPERNATANT FLUID ACTIVITY	PRECIPITATE ACTIVITY
				%	%
1	Cerebellar cortex	20	11.28	19	81
1	" "		12.73	24	70
2	" "		6.22	37	66
3	" "		8.47	29	72
Average...			9.68	27	72
1	Caudate nucleus	40	43.81	20	80
2	" "		23.38	28	72
Average...			33.59	24	76
1	Thalamus	40	5.59	44	64
2	"		5.01	34	64
Average...			5.30	39	64
1	Cerebellar medulla	70	8.33	31	74
3	Medulla	80	6.78	43	65
2	"		4.47	39	73
Average...			5.62	41	69
1	Spinal cord (C1-C3)	85	3.63	55	49
1	Internal capsule	100	2.59	44	66
1	White matter	100	0.93	47	60
1	Cerebellar peduncle	100	6.38	49	58
1	Corpus callosum	100	0		
2	" "		0		

supernatant fluid fraction is suggestive that this fraction may be more highly concentrated in the fiber or some portion of it and that its physiologic role may be related to this distribution.

#### SUMMARY

Dog brain homogenates have been shown to contain two fractions of cholinesterase similar to the previous finding with mouse brain. The fraction present in the precipitate obtained by centrifuging the homogenate is more heat labile than is the supernatant fluid fraction. The  $Q_{A \cdot Ch}$  values for various portions of the dog

brain are as follows: caudate nucleus 33.6, cerebellar cortex 9.7, cerebellar medulla 8.3, cerebellar peduncle 6.4, medulla 5.6, thalamus 5.3, spinal cord 3.6, internal capsule 2.6, white matter 0.93, corpus callosum 0.

In general, there is a direct relationship between the estimated relative mass of nerve fiber in the tissue and the activity of the supernatant fluid fraction of cholinesterase. With the exception of the corpus callosum, this relationship is suggestive that the supernatant fluid fraction may be more highly concentrated in the nerve fiber.

The author wishes to acknowledge with appreciation the assistance of Miss Earline Tapp in many of these experiments.

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# THERMAL REACTIONS OF YOUNG ALBINO RATS TO INTRAPERITONEAL INJECTIONS OF ERGOTOXINE<sup>1</sup>

A. R. BUCHANAN AND J. E. ROBERTS

*From the Department of Anatomy, University of Colorado Medical Center*

DENVER, COLORADO

**E**RGOTOXINE, administered intraperitoneally to albino rats over 30 days of age, has been found to be productive of hyperthermia if the animals are in a comfortably warm environment (1). The prevailing concept that ergotoxine has a central action has seemed to provide the most logical explanation for these phenomena.

Prior to the attainment of an age ranging between 18 and 30 days, albino rats are unable to maintain their body-temperatures when placed in a cold environment (2, 3). If the attainment of the ability to maintain a normal body temperature when exposed to adverse environmental situations depends upon the maturation of the hypothalamus (3) and if the thermal responses to ergotoxine are dependent upon hypothalamic stimulation, it seemed logical to hypothesize that pre-regulatory rats might fail to react to ergotoxine in the same manner as the older animals previously reported (1). The research presently reported was designed to test this hypothesis.

## MATERIALS AND METHODS

Seven litters of Wistar strain albino rats have been used in the accumulation of the data specifically reported herein. A great many additional observations upon rats of comparable ages, but from other litters, have been made in connection with other studies; the thermal reactions of these animals have been similar, in all respects, to those presented.

Four rats from one litter (2J) were used as ergotoxine experimental animals and the remaining 3 served as controls. Ergotoxine ethanesulphonate,<sup>2</sup> dissolved in 6.25 per cent ethyl alcohol (1 mg. in 2 cc.), was administered intraperitoneally to the experimental rats in a dosage of 4.5 mg/kg. of body weight. Each animal received the drug on at least 8 separate occasions such that its thermal reactions were studied at intervals ranging from 12 to 53 days of life. Continuous temperature records were obtained concurrently from one experimental and one control animal by means of copper-constantan thermopiles and a two-channel Brown electronic potentiometer as previously described (1, 3).

The members of the other 6 litters were each given ergotoxine only once, at ages ranging from 12 to 32 days. Immediately after administration of the drug to the experimental animals in litters 3N and 3R (fig. 3), each, together with a litter-mate.

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Received for publication August 23, 1948.

<sup>1</sup> This research was supported by the Office of Naval Research.

<sup>2</sup> Kindly furnished by the Wellcome Research Laboratories, Tuckahoe, N. Y.

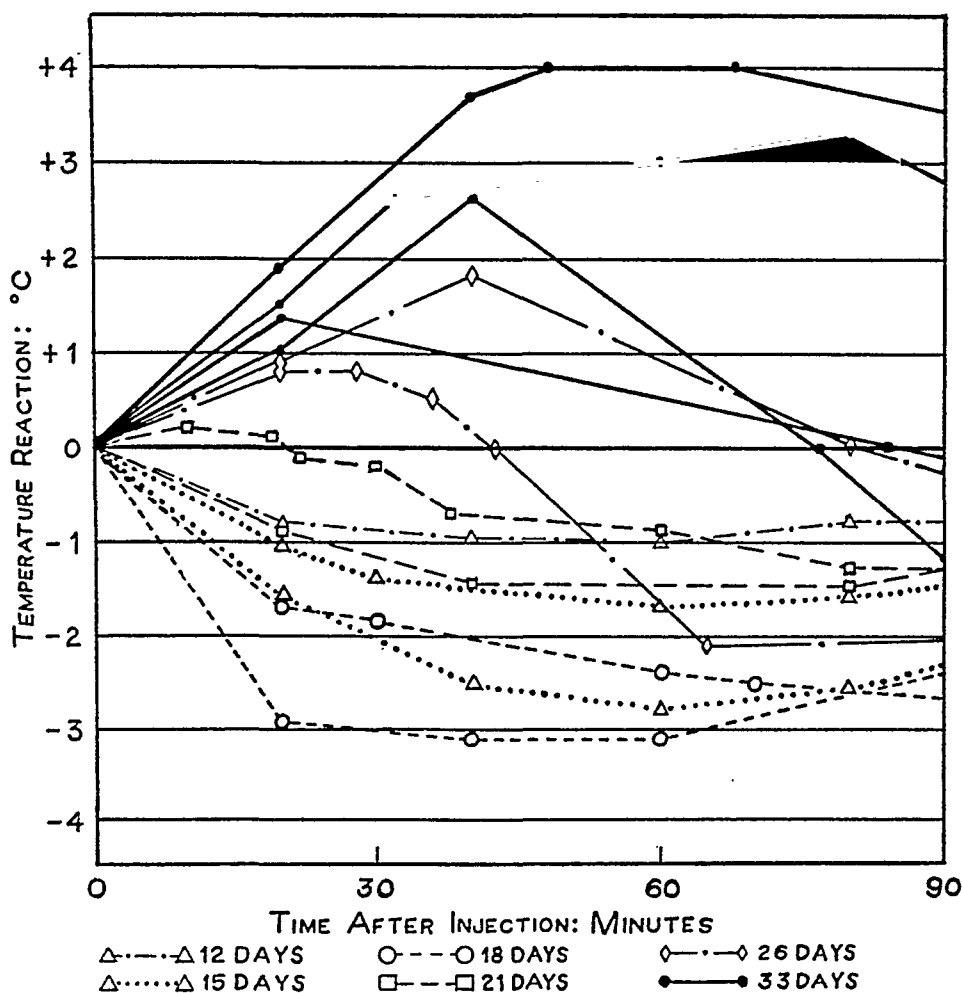


Fig. 1. THERMAL RESPONSES of litter-mate rats (*litter 2J*) given single intraperitoneal injections of ergotoxine ethanesulphonate (4.5 mg/kg.) on successive occasions and at the ages indicated.

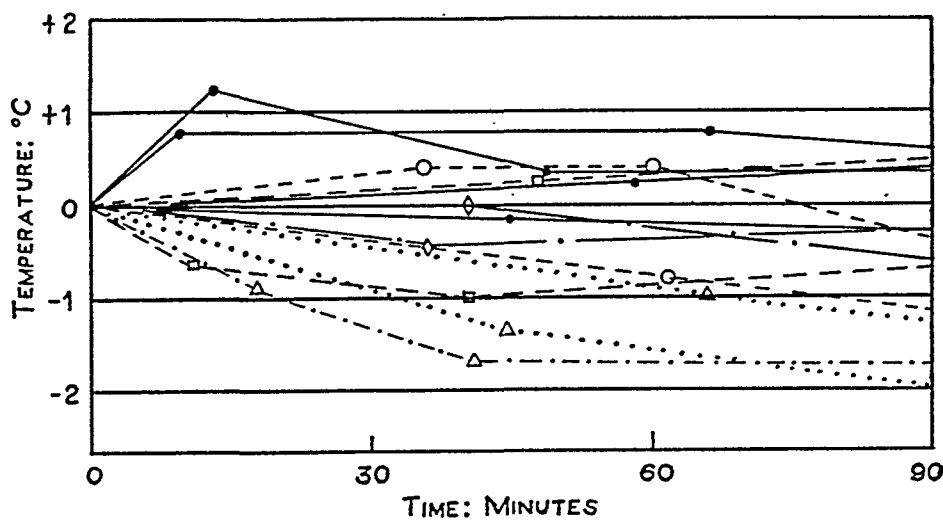


Fig. 2. TYPICAL TEMPERATURE RECORDS of litter-mate controls of experimental rats used in the compilation of figure 1. The legend accompanying figure 1 also applies to this figure.



cated in figure 2 and are obviously much less pronounced than the reactions in ergotoxine-treated animals. The 12- and 18-day rats exhibited hypothermia in response to ergotoxine which was more pronounced at 18 than at 12 days. Hyperthermic responses were well developed at 26 days of age and pronounced at 33 days. Beyond the 33rd day the responses continued to be of considerable magnitude and on the side of hyperthermia.

Rats given single injections of ergotoxine at ages ranging from 12 to 32 days responded in the same manner as those receiving successive injections at corresponding ages. The thermal reactions of the members of 6 litters are plotted as degree-minutes in figure 3. With the exception of the 12-day rat from *litter 3N*, all the 12- and 16-day animals exhibited hypothermic responses.

Hyperthermia appeared on the 20th day in *litters 3N*, *3R*, and *3Z*, on the 23rd day in *litter 3Y*, on the 26th day in *litter 4F*, and on the 28th day in *litter 4G*. The temperatures of all the animals in these 6 litters were recorded for 2 hours, but the hyperthermic areas, as plotted in figure 3, include only the initial responses. Hypothermias, which regularly follow the primary hyperthermic reactions, were not summed with the latter. As was true in individual members of *litter 2J* (fig. 1), the duration of the initial response at a given age varied considerably from litter to litter.

Litter-mate controls from *litter 4G*, studied under identical conditions except that they did not receive ergotoxine showed only minor fluctuations in temperature.

#### DISCUSSION

The fact that ergotoxine failed to elicit hyperthermia in young (pre-regulatory) rats (with one exception) appears to be related to the inability of animals of the same strain and within the same age range to maintain their body-temperatures when placed in a cold environment (3). If ergotoxine hyperthermia is due to direct action of the drug on the hypothalamus (4, 5), its failure to appear in young rats seems to indicate a lack of maturity of this area of the brain and the results presented above may be considered as strongly supporting our previous contention that the attainment of regulatory ability is dependent upon maturation of the hypothalamus and its descending connections (3).

The hypothermic reactions to ergotoxine of the young rats were unexpected and are not easily explained. It is possible that they are due in part, at least, to peripheral vasodilatation associated with sympathetic reversal as described by Dale (6) and that the hypothermia in 16- and 18-day animals is more pronounced than in those 12 days of age because of a greater degree of maturity in the peripheral sympathetic mechanisms of the older rats. If this hypothesis is accepted, it is necessary to assume further that, upon maturation of the central regulatory mechanism in the hypothalamus, the more primitive peripheral response to ergotoxine is superseded by it.

The hyperthermic responses to ergotoxine are usually accompanied by considerable muscular activity and the hypothermic response (in the immature animals) by an almost anesthetic quiescence. It is worthy of note, however, that the 26-day rat in *litter 4G* displayed increased muscular activity in the presence of hypothermia; this, together with other similar observations, suggests that the hyperthermic reactions are only partially due to the effect of ergotoxine on muscular activity.

## SUMMARY

The hypothesis that thermal responses to ergotoxine of rats in the pre-regulatory age range might differ from those of regulatory animals has been experimentally verified. It has been found that rats 12 to 18 days of age usually react to ergotoxine with varying degrees of hypothermia as contrasted to the reactions of their older litter-mates (20-30 days), which, under identical environmental conditions, are almost uniformly on the side of hyperthermia. Variations from litter to litter, so far as the age at which reversal of the response occurs, are common. Such variations have also been observed with regard to the age at which attainment of the ability to regulate body temperature occurs. The age range of reversal of the ergotoxine reaction (from hypo- to hyperthermia) and that at which regulatory ability is attained closely coincide. These observations lead to the conclusion that both phenomena depend upon the maturation of the hypothalamus.

The authors thank Dr. R. W. Whitehead, Professor of Physiology and Pharmacology, University of Colorado Medical School, for valuable suggestions and criticism.

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# EXPERIMENTAL MYOTONIA AND REPETITIVE PHENOMENA: THE VERATRINIC EFFECTS OF 2,4-DICHLORPHENOXYACETATE (2,4-D) IN THE RAT<sup>1</sup>

C. EYZAGUIRRE, B. P. FOLK, K. L. ZIERLER AND J. L. LILIENTHAL, JR.

With the technical assistance of E. LEAKINS

*From the Physiological Division, Department of Medicine, The Johns Hopkins University and Hospital*

BALTIMORE, MARYLAND

THE profound botanical effects of certain substituted phenoxyacetic acids have been described by several investigators of plant growth (1, 2). A familiar example of these growth-regulating agents is 2,4-dichlorophenoxyacetic acid (2,4-D); but over one thousand compounds, directly or remotely related, have been examined and many were found to exert similar effects on plants (3).

Certain extraordinary effects of 2,4-D were noted in representative mammals by Bucher (4). Most striking was the development of muscular rigidity which appeared to be myotonic in character and was reported to be so established by myographic and electromyographic analysis.

This communication reports certain effects of 2,4-D on the neuromuscular system of the rat mimicking the myotonia which occurs spontaneously in man (5) and in the goat (6-8). These effects were indistinguishable from those produced by a large and apparently unrelated group of chemical and physical stimulating agents.

## METHODS

The rats were of both Whelan and Sherman strains, weighing 140 to 400 g. The triceps surae and sciatic nerve and its tibial branch were studied exclusively. Anesthesia was induced by sodium pentobarbital (Nembutal). Skin and tissue overlying the sides of the distal end of the femur were split for approximately 5 mm. The underlying femur was fixed rigidly by a clamp designed on the principle of a micrometer; the shaft of the femur was held between the advancing spindle and anvil, and the sleeve was fixed to the myograph frame. A short incision over the heel permitted separation of the tip of the calcaneus with its attached tendon. The muscle, when prepared for recording, enjoyed an undisturbed blood supply and was covered with intact skin. The tendon was transfixed by and secured to a short hook of piano wire (No. 20 B. & S. gage) which was attached to a cylinder of bakelite for insulation. The upper end of the bakelite cylinder was screwed to the armature pin of a Statham YE-48-600 strain gage.<sup>2</sup> The strain gage was mounted rigidly to the main column of a Brown-Schuster myograph. This type of strain gage is particularly adapted

Received for publication August 27, 1948.

<sup>1</sup> Work done under a contract between the Office of Naval Research, U. S. Navy Department, and the Johns Hopkins University.

<sup>2</sup> Statham Laboratories, Los Angeles, Calif.

for isometric recording; the movement of the pin under a full load of 1500 g. is limited to 0.04 mm. The bridge signal was fed through a direct-coupled amplifier to a cathode ray oscilloscope for photographic recording. The bridge output was linear through full-scale deflection. Resting tension of the muscle was maintained at a constant value throughout any one experiment, but varied from one experiment to another between 75 and 200 g.

For indirect stimulation the sciatic nerve was crushed and tied proximally. The distal segment was placed in a plastic insulating bed containing two silver stimulating electrodes. Direct stimulation was effected through steel needles placed at opposite ends of the muscle. Stimuli, which were supramaximal in intensity, were presented as square waves generated by a trigger circuit, the duration of which was controlled by a variable resistance-capacitance circuit.

Nerve action potentials were recorded through a Sherrington electrode from the tibial nerve isolated peripherally. Stimulation was effected at the pelvic end of the sciatic nerve which was isolated centrally.

Electromyograms were recorded through steel needles insulated to within 1 to 2 mm. of their tips and placed in the belly of the muscle approximately 5 mm. apart. The potentials were fed through a condenser-coupled amplifier to the cathode ray oscilloscope and recorded photographically. When the needles were inserted shortly after section of the sciatic nerve, spontaneous random spike potentials were observed. These spikes lessened in frequency and then disappeared in the course of 10 to 15 minutes. No studies were carried out until spontaneous activity had subsided.

Intra-arterial injection was made through a small cannula tied into the contralateral iliac artery and directed centrally. The crural artery of the leg under study was ligated. The aorta was occluded during injection by drawing up on a loose ligature placed above the bifurcation. The injection volume was 0.15 ml. This preparation preserved normal blood supply to the muscle under study but is under continuing development to attain further restriction of the injection to the triceps surae. The small size of the rat's peripheral vessels enabled the use of a simple high-frequency desiccating apparatus ('Hyfrecator', Bircher) to coagulate vessels and, thus, to reduce bleeding.

When required, denervation of the triceps surae was performed 10 days before recording by aseptic section of the sciatic nerve.

Concentrations of potassium in serum and muscle were determined in control rats of the same weight in whom equivalent amounts of KCl had been injected intraperitoneally (i-p). Analyses were made in a modified Berry-Chappell-Barnes internal standard flame photometer (9, 10).

Prior to administration of curare the trachea was cannulated and artificial respiration provided by a pump.

The concentrations of the agents used and their dosages follow:

- 1) Sodium pentobarbital, 25 mg/ml., was administered i-p in doses of 40 to 50 mg/kg. body weight.
- 2) Sodium 2,4-dichlorphenoxyacetate monohydrate (Baker) was dissolved in water in a concentration of 50 mg/ml., and the pH was adjusted to 7.4 with dilute HCl for i-p injection in doses of 100 to 250 mg/kg.

3) *d*-Tubocurarine chloride<sup>3</sup> was injected i-p in doses of 1.5 mg/kg. This is equivalent approximately to one unit of curare per animal and produced total paralysis of all skeletal muscle.

4) KCl was injected i-p as a 20 mg/ml. (270 mEq/l.) aqueous solution. Doses were 400 mg/kg. (5.4 mEq.).

5) Quinine dihydrochloride was injected intramuscularly in aqueous solution of 45 mg/ml. and in doses of 150 mg/kg.

6) Disodium *d*-l- $\alpha$ -tocopheryl phosphate<sup>4</sup> was injected i-p in an aqueous solution of 100 mg/ml. in doses of 1 g/kg.

7) Magnesium sulfate was injected i-p in doses of 250 mg/kg.

8) Calcium gluconate was injected i-p in doses of 100 mg/kg.

## RESULTS

Our observations are in full agreement with those of Bucher on the behavior of the rat which has received 2,4-D (4). In both conscious and anesthetised rats full development of myotonia appeared some 30 to 45 minutes after i-p injection and lasted for hours. The injection of small amounts (2 mg.) of 2,4-D into the vascular tree was followed by extreme generalised myotonia in two minutes. Even under deep anesthesia and full curarisation, the resistance to passive motion of the extremities was appreciably enhanced.

Primary effects of 2,4-D on muscle function are illustrated in figures 1 and 2. Increase in tension developed by an isometric twitch, in response to a single supra-maximal stimulus to the nerve, was of the order of 25 to 30 per cent; duration of twitch until half relaxation was greatly prolonged (figs. 1A and 2A).

The electromyogram of normal muscle stimulated by a single shock to the nerve is a simple diphasic deflection (fig. 1B). When myotonia had developed after injection of 2,4-D the response became repetitive. The rapidly recurring, brief potential changes were less than the initial spike, and occasionally they did not appear until after a short period of electrical silence, 10 to 50 msec., following the initial spike. On one occasion the silence persisted for 600 msec. (fig. 3C). In some records the regular rhythmicity of the repetitive response suggested that a single motor unit was firing directly under the recording electrodes. The duration of repetition was variable, ranging from 100 msec. to 6 sec.

Prolongation of twitch and repetitive firing decreased rapidly with repeated stimulation; at a stimulus rate of 12 per min. myotonic features diminished rapidly during the first five or six consecutive single volleys (fig. 1A, B). Rest for 10 minutes resulted in complete return of the myotonic response. The same changes were noted following stimulation by a pair of nerve volleys delivered at short intervals.

Myotonia in man and goat is characterised by exquisite sensitivity of muscle to mechanical stimuli. 2,4-D produced in the rat this same explosive electrical response to tapping of the muscle or tendon and also to the insertion or movement of the recording electrodes (fig. 2).

The site of development of the myotonic response to 2,4-D was delimited partially by injection of sufficient *d*-tubocurarine chloride to block completely any muscle

<sup>3</sup> Generously provided by E. R. Squibb and Sons, New York, N. Y., and by Abbott Laboratories, North Chicago, Ill.

<sup>4</sup> Generously provided by Hoffman-LaRoche, Nutley, N. J.



response to indirect stimulation. Under these conditions, direct electrical or mechanical stimulation of curarized muscle produced increased twitch tension, protracted relaxation time and repetitive response indistinguishable from that observed in non-curarized muscle (fig. 3). The triceps surae, denervated 10 days before examination, also developed myotonia in response to 2,4-D despite obvious atrophy.

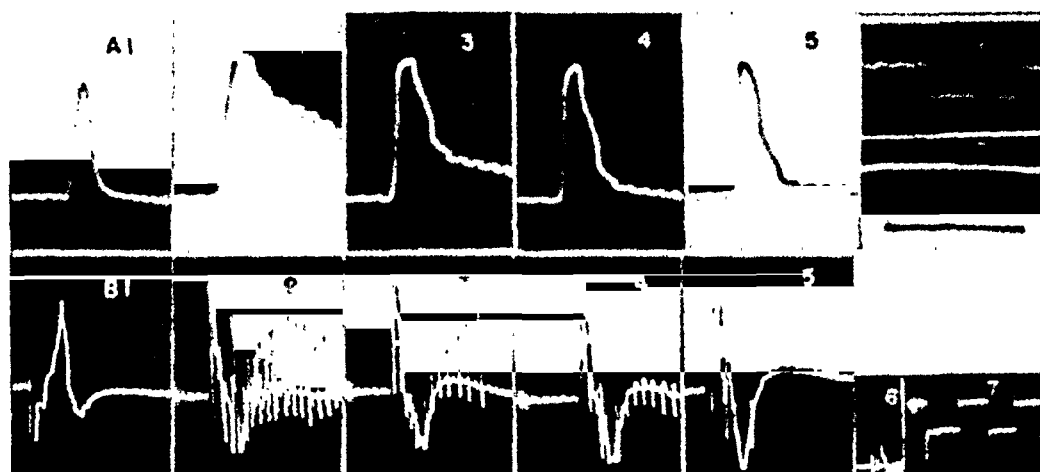


Fig. 1. INDIRECT STIMULATION OF THE TRICEPS SURAE BY THE SCIATIC NERVE. A. 1. Normal myogram. 2-5. Consecutive myograms, 120 min. after 40 mg. of 2,4-D, in response to stimuli delivered at a rate of 12/min., illustrating decreased duration and the phenomenon of 'warm-up'. 6. Time: 50 msec. 7. Tension: 200 g. B. 1. Normal electromyogram. 2-5. Consecutive records as in A. 6. Voltage: 200  $\mu$ V. 7. Time: 50 msec.

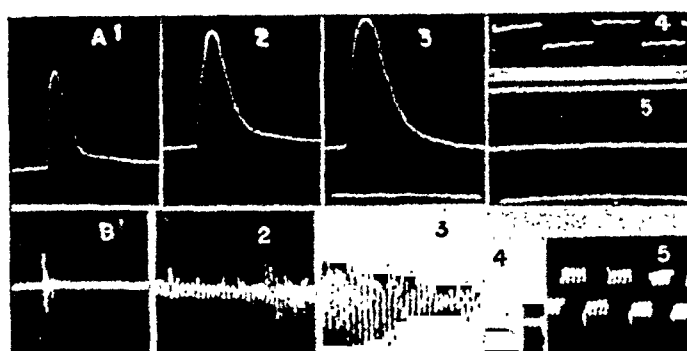


Fig. 2. INDIRECT STIMULATION AND MECHANICAL TAPPING. A. 1. Normal myogram. 2. 30 min. after 30 mg. 2,4-D. 3. 39 min. after 60 mg. KCl (same preparation as 2). 4. Time: 50 msec. 5. Lower line: zero tension; middle line: resting tension of 143 g.; upper line: 200 g. B. Electromyogram in response to a brisk tap on tendon. 1. Normal. 2 & 3. Corresponds to A2 and 3. 4. Voltage: 1  $\mu$ V. 5. Time: 50 msec.

The familiar simple diphasic electrogram of nerve stimulated by a brief single shock is recorded in figure 4 (11). One hour after administration of 2,4-D a similar single stimulus evoked a volley of potentials which arose in the nerve proper, since it had been isolated from both central and peripheral structures.

Certain agents which modify spontaneous myotonia were studied for their effects on the myotonia produced by 2,4-D. *Potassium*, 5.4 mEq/kg. i-p, elevated the serum concentration from a normal level of 5 mEq/l. to 10 to 14 mEq/l. in 20 to 30

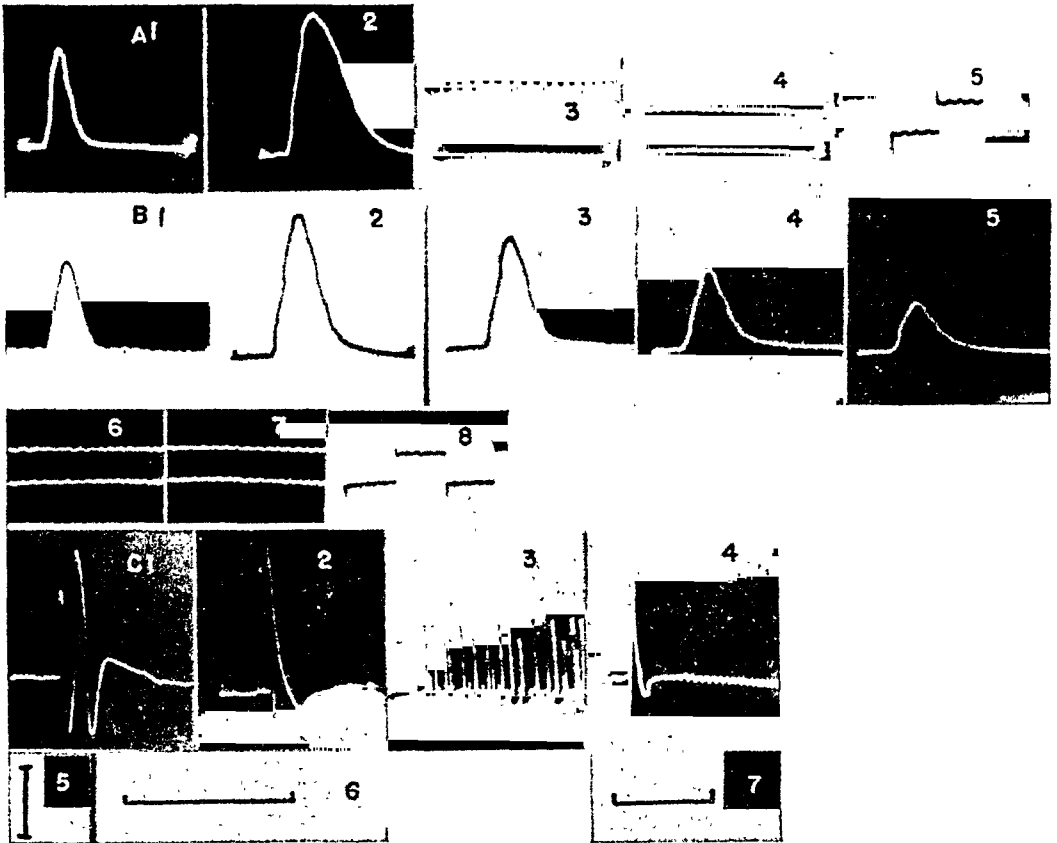


Fig. 3. COMPLETELY CURARIZED PREPARATION; DIRECT STIMULATION. A. 1. Normal myogram 2. 45 min. after 40 mg. 2,4-D. 3. Tension: 200 g. 4. Resting tension: 125 g. 5. Time: 50 msec. B. 1. Normal myogram. 2. 45 min. after 40 mg. 2,4-D. 3. 11 min. after 20 mg. quinine. 4. 5 min. after additional 20 mg. quinine. 5. 10 min. after 4. 6. Tension: 200 g. 8. Time: 50 msec. C. 1. Normal electromyogram. 2 & 3. Consecutive sweeps 40 min. after 30 mg. 2,4-D, illustrating an unusually prolonged period of silence (600 msec.). 4. 5 min. after 40 mg. quinine (irregularity of trace is owing to 120 cycle interference). 5. Voltage: 1  $\mu$ V. 6. Time: 50 msec. (referring to 1-3). 7. Time: 50 msec. (referring to 4).

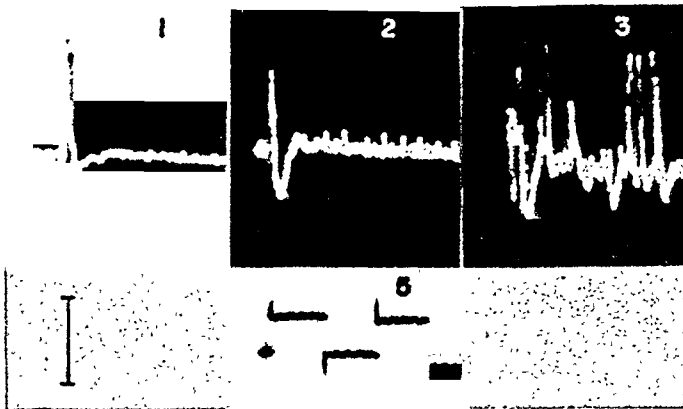


Fig. 4. DIPHASIC ACTION POTENTIAL FROM TIBIAL NERVE. 1. Normal. 2. 15 min. after 30 mg. 2,4-D. 3. 60 min. after 2,4-D. 4. Voltage: 200  $\mu$ V. 5. Time: 50 msec.

minutes. This increase in serum concentration presumably was reflected rapidly in the interstitial fluid surrounding the muscle cells, but analyses of the total muscle itself revealed no significant increase in total K content. We have confirmed

Walker's findings that in the normal rat this distortion of  $K^+$  relationships resulted in a heightened and lengthened tension response to both direct and indirect stimulation and in repetitive firing of the muscle in response to a single stimulus (12). The administration of KCl to a rat in full course of response to 2,4-D produced a further potentiation of the myotonic response and in degree of repetitive firing (fig. 2B).

Increased sensitivity to *acetylcholine* was demonstrated by injecting 100  $\mu$ g. intra-arterially (11). In the preparation described here, this injection elicited a few small scattered spike potentials in the electromyogram. After treatment with 2,4-D the same amount of acetylcholine evoked an explosive and prolonged burst of electrical activity.

*Quinine* has been shown to reduce or obliterate the myotonic response occurring spontaneously in man (13, 14) and goat (6, 8). The same effect was produced on myotonia induced in the rat by 2,4-D. The myogram revealed a lowering of tension following administration of quinine (fig. 3B). There are certain characteristics of this response which warrant noting. First, tension developed after the second dose of quinine fell to 54 per cent of the control twitch; and second, the duration of the twitch was not reduced. The effect of quinine on the responses induced by 2,4-D is shown in figure 3C where the characteristic repetitive firing of the muscle was obliterated.

*Alpha-tocopheryl phosphate*, when injected i-p in relatively large doses into normal rats, produces profound generalized effects: somnolence, ataxia, muscular flaccidity, coma and, occasionally, death following convulsions (15). When administered per-orally to two patients with myotonic dystrophy a suggestive decrease in spontaneous myotonia was observed (16). Injection of  $\alpha$ -tocopheryl phosphate in the rat, which had received 2,4-D, obliterated the evidences of myotonia as effectively as did quinine.

Similar suppression of repetitive response followed injection of *magnesium* and *calcium*.

#### DISCUSSION

The neuromuscular apparatus of the rat, treated with 2,4-D, mimics faithfully the characteristics of myotonia occurring spontaneously in man and goat. In both circumstances, muscle exhibits exquisite sensitivity and repetitive response to several stimulating agents: motor nerve volley and direct stimulation, tapping, insertion of needle electrodes, acetylcholine and  $K^+$  (6). Furthermore, in both instances, repeated stimulation ('warm-up'), quinine,  $Mg^{++}$  and  $Ca^{++}$  diminish and obliterate the repetitive phenomenon. Alpha-tocopheryl phosphate now may be added to the list of agents inhibiting repetitive responses in both spontaneous and induced myotonia.

These effects of 2,4-D, producing a myotonic reaction so reminiscent of the spontaneously occurring forms, are indistinguishable, likewise, from the consequences of treatment with a variety of substances; e.g., the 'veratrine' alkaloids derived from *Veratrum* and *Schoenocaulon*, aldehydes, tetraethyl ammonium ions, phenanthrene-9-carboxylic acid and substituents, and dihydronaphthacridine carbonic acid

(Tetrophan) (17). To this heterogeneous group we have added recently pentamethylene tetrazol (Metrazol) (18).

Many, and perhaps all, of these diverse veratrinic substances, however, produce the same repetitive response in nerve that they produce in muscle. It has not been established in spontaneous myotonia whether or not this repetitive phenomenon occurs in nerve as well as in muscle; nevertheless, some observations have suggested that myotonia in man is accompanied by functional changes not limited to muscle fiber proper (19). Indeed, it is reasonable to view repetition as a stereotyped response of excitable tissue (nerve, neuromuscular junction and muscle) to many unrelated alteratives which may play rôles of differing intensity on all excitable structures. A tentative exploration of this possibility might begin by examining the circumstances in which repetition has been observed.

In order to limit the analysis, only those instances of repetitive response to single stimuli have been considered; obviously, this limitation excluded other important changes in excitability manifested by spontaneous firing or by measurable changes in pre-discharge conditions, such as threshold, resting potential, recovery cycle etc. (20, 21). The appearance in excitable tissue of repetitive, rhythmic responses to a single stimulus occurs under the most diverse of circumstances, as for example: *a*) increased external hydrostatic pressure (22); *b*) stimulation by constant rectangular currents (23); *c*) increased external concentration of  $H^+$  (20, 24),  $K^+$  (12), acetate, lactate, citrate and oxalate (24),  $Ba^{++}$  and guanidine (25), adenosine triphosphate (26), DDT and quinoline (27); *d*) decreased external concentration of  $Ca^{++}$  (28); *e*) inhibition of cholinesterase activity at the neuromuscular junction by eserine (11), neostigmine (29) and DFP (30); *f*) tetanus toxin affecting the neuromuscular junction (31); *g*) treatment with that heterogeneous group of substances which produce veratrinic effects (17); and *h*) spontaneous myotonia in man and goat.

In the face of our fragmentary knowledge of processes subserving excitability, the incongruity of those conditions and agents producing repetitiveness makes it extremely difficult to apply any unifying concept which can explain all of the observations noted above. An example of the difficulties encountered in a simple approach is contained in an attempt to analyse the phenomenon of repetitiveness in terms of distortions of cationic *milieu*. Preservation of totally normal function depends on maintenance of concentrations of  $Na^+$ ,  $K^+$  and  $Ca^{++}$  within certain limits. Could these many agents act by disturbing the required cationic patterns? Simple inspection of the structure of the veratrinic agents reveals their variation through almost full scale with respect to chemical activity, steric and physical characteristics; some are potential metal sequestrants or precipitants, others totally inactive. Lack of any common characteristic is emphasized not to imply that a unitary pattern of action is non-existent but to stress the necessity for fitting many divergent observations into any proposed scheme.

It seems likely that these many different forces and agents may act at different loci in the complicated train of events responsible for the smoothly integrated flow of energy which maintains normal excitability. Only vague clues exist to suggest certain possible sites of action.

For example Lorente de Nô, from an elaborate analysis in frog nerve, suggests

that, "Veratrine by reducing the speed of or blocking some late step in the chain of oxidative processes interferes with the maintenance of the membrane potential. . . ." (24). From a study of plants and micro-organisms comes suggestive evidence that 2,4-D distorts mechanisms involved in the transfer of oxygen (32, 33). Compatible with, but not proving, this suggestion is the suppressive action of  $Mg^{++}$  on veratrinic phenomena (24) and the similar action of  $\alpha$ -tocopheryl phosphate which is so potently antioxidative as well as antiproteolytic (15).

Models of excitability or energy transfer expressed in terms of chemical or physical structure, enzymatic activity, surface activity or membrane potential, may explain some or many of the observations; but none, as yet, has been offered in satisfactory interpretation of all the data.

The phenomenon of the silent period intervening between the normal single spike potential and the outburst of repetitive spikes following treatment with 2,4-D is the same as that described by Eichler in the frog treated with small amounts of 'veratrine' (34). A speculative interpretation of this phenomenon might assume exponential decay of facilitating and depressing processes initiated simultaneously by the stimulus (35). If under the observed circumstances the depressing process decayed so rapidly that the facilitating process remained unopposed, then a burst of activity might be released. The data available do not permit a more specific analysis.

#### SUMMARY

2,4-dichlorophenoxyacetate produces in the neuromuscular apparatus of the rat a veratrinic response marked by repetitive response to single stimuli in muscle and nerve. This results in increased 'twitch' tension and prolonged 'twitch' duration. The repetitive responses and their sequelae are accentuated by  $K^+$  and acetylcholine and are obliterated by activity, quinine,  $Mg^{++}$ ,  $Ca^{++}$  and  $\alpha$ -tocopheryl phosphate. These phenomena are indistinguishable from those occurring in the spontaneous myotonia of man and goat, and also in response to several apparently unrelated chemical, physical and electrical agents.

We are deeply indebted to Dr. S. A. Talbot for designing and supervising construction of the electronic instruments used in these studies.

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# EFFECT OF MECHANICAL VIBRATION ON THE PATELLAR REFLEX OF THE CAT<sup>1</sup>

DAVID E. GOLDMAN

*From the Naval Medical Research Institute*

BETHESDA, MARYLAND

IN 1938 Coermann (1) surveyed some of the physiological effects of the exposure of man to mechanical vibration in the frequency range 20 to 1000 cps. One of the few positive results obtained was that, during the operation of a vibrating platform on which a human subject was seated, it was difficult or impossible to obtain the patellar reflex. This phenomenon was further investigated by Loeckle (2). Briefly, the latter found that the application of a vibrating rod to the skin area over the course of the femoral artery, vein and nerve of a human subject produced abolition or reduction of the reflex, whereas if the rod was applied directly to the quadriceps muscle, no inhibitory effect was found. Further, in one experiment on an anesthetized cat, he found that application of the vibrator to the femoral artery which had been lifted free from the surrounding tissues for a distance of a few cm. abolished the reflex. When the artery was stripped of its adventitial layer, the inhibitory effect vanished. In addition, he studied an individual on whom a unilateral lumbar sympathectomy had been performed one year before. On the sympathectomized side only it was found impossible to inhibit the patellar reflex by vibration. He used frequencies from 30 to 100 cps. and amplitudes up to about one mm. Loeckle naturally concluded that the inhibition was mediated by the sympathetic fibers of the periarterial plexus and the sympathetic ganglionic chain.

Echlin and Fessard (3) applied a vibrating tuning fork (85-530 cps.) or a vibrating steel strip (5-32 cps.) to a muscle or its bony support, and recorded nerve action potentials. The potentials tended to be synchronized with the vibrating stimulus. Sommer (4) obtained electromyograms from the biceps of the vibrated arm of a man (37 cps.). These potentials were also synchronized with the vibration. On the other hand, Loeckle's electromyograms of the human quadriceps showed no such impulses.

In view of these observations, the rôle of the autonomic nervous system in mediating the reflex inhibition seems doubtful. The intrinsic interest of these phenomena, together with their practical importance in relation to human exposure to vibration, justified further experimental work.

## MATERIAL AND METHODS

Successful experiments were carried out on 7 cats. Two of these experiments also involved decerebration and two more, lumbar sympathectomies. The animals were anesthetized with ether and the quadriceps femoris was exposed together with its tendon, nerve and vascular supply, using

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Received for publication July 15, 1948.

<sup>1</sup> The opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

great care not to damage these structures. The nerve supply to antagonistic muscle groups was severed.

Mechanical vibration was applied with a permanent magnet loud speaker from which the cone had been removed and to which a rod 6 inches long by  $\frac{1}{4}$ -inch diameter had been fixed. The device was driven by an audio oscillator and a 15-watt public address system amplifier. It was mounted on an adjustable stand over the animal operating board so that it could be lowered vertically into contact with the part to be stimulated. The vibrator also could be applied manually in other directions. Observations of the wave form of the vibrator indicated roughly 10 to 20 per cent harmonic content. Frequencies of 10 to 600 cycles per second were used. The maximum amplitude obtainable at the lower frequencies was about 1 millimeter, with less at the higher frequencies. For most purposes, 100 cycles and about 1-millimeter amplitude were used. The patellar reflex was elicited by regular, controlled tapping of the tendon.

### RESULTS

When the vibrator was applied directly to the belly of the muscle or to the tendon or the femur, the knee jerk was much reduced or was abolished entirely. The effect appeared immediately upon applying the vibrator and disappeared immediately on removing it. The muscle was often seen to undergo a slight contraction which persisted as long as the vibrator was applied. Application of the vibrator to the artery *in situ* occasionally produced a very slight diminution of the reflex. However, when the artery was lifted free from the underlying structures for a distance of a centimeter or so, no inhibitory effects could be obtained.

Two cats were also decerebrated and further prepared to observe contraction of the quadriceps by elicitation of the crossed extensor reflex (stimulation of the central cut end of the opposite sciatic nerve). During application of vibration, with the patellar reflex inhibited, the crossed extensor reflex was easily obtained. In another experiment, after obtaining the reflex responses and their inhibition by vibration, the animal was subjected to a bilateral lumbosacral sympathectomy. This procedure had no observable effect on either the patellar reflex or its inhibition by vibration. Subsequent stripping of the adventitia from about a centimeter of femoral artery just below the fossa ovalis left both the reflex and its inhibition unchanged. In another cat a unilateral sympathectomy was performed and both thighs examined as described above. No difference in either the reflex or its inhibition was found between the two sides.

It is evident from the above findings that the inhibitory action of vibration need have no direct dependence on the presence of sympathetic pathways. The likelihood is, therefore, that the inhibition is only apparent. The vibration itself may stimulate the reflex periodically and thus make it very difficult for a superimposed stimulus to be effective. The tapping of the tendon is apparently a stimulus of too great duration to be effective in competition with vibratory stimulation at 100 cycles per second. However, stimulation at frequencies low enough to give a period longer than the duration of a tap should permit at least occasional tapping stimuli to produce a reflex. In order to test this, the vibrator was applied at frequencies of 10, 15, 20, and 30 cycles. It was then observed that with 10-cycle vibration most of the tendon taps produced a response. At 15 cycles only about one half of the taps produced a response. At 20 cycles, roughly one-quarter were effective, and at 30 it was only rarely possible to obtain the reflex contraction by tapping.



In addition, the effects of increasing the frequency of vibration beyond 100 cps. were observed. In three experiments it was found that the vibration became ineffective as an inhibitory agent at frequencies of from 300 to 600 cycles per second. It is not clear how far this results from a falling off of the amplitude and how far it depends on frequency characteristics of the sensory receptors.

#### DISCUSSION

The above experiments are consistent with the following interpretation. Vibration produces a periodic, synchronous stretch reflex in which many of the muscle receptors are involved. There is thus a slight continuous contraction of the muscle and tapping the tendon is now ineffective since the reflex arc is in steady use. However, this does not involve all of the motor neurons since muscular contraction is obtainable via other routes, in this case the crossed extensor pathways. Also, it is generally accepted that a rôle of the sympathetic nervous system is to enhance rather than to inhibit muscular activity. As far as the specific reflex studies here are concerned there is at this time little evidence of any direct intervention of the autonomic nervous system although it is known (5) that stretch and other muscular reflexes may be affected by autonomic stimuli. One is inclined to interpret Loeckle's findings on the cat as artifacts referable to the practical difficulty of handling the vibrator and to the mechanical transmission of the vibratory motion from the artery to the muscle. Also, he used a fixed anesthetic (pernoston) which may have had a depressant effect on the reflex mechanism. His observations on the human sympathectomized subject, however, still require explanation. There is no evidence available at present for elucidating this.

The phenomena described here are of some practical interest in connection with the exposure of human beings to vibrating machinery etc., or to very intense sound fields. A number of unconfirmed incidents have at various times been reported in which such exposures apparently produced muscular weakness, especially in the lower limbs, and even led to collapse, without, however, any injury occurring. The above observations suggest that there may well be a genuine physiological basis for these experiences. Further work will have to be done before any quantitative relationship can be established between the phenomena and the mechanical forces producing them.

#### SUMMARY AND CONCLUSIONS

Experiments on the effects of mechanical vibration on the patellar reflex of the cat confirm previous observations that such vibration produces an inhibition. It is shown that the inhibitory phenomenon is readily explained on the basis of vibratory excitation of the reflex itself. The autonomic nervous system plays no direct part in the phenomenon. The inhibition appears between 10 and 30 cps. and fades out gradually above 300 cps. with the apparatus used. It is suggested that the inhibitory phenomenon may underlie certain effects of the exposure of man to mechanical vibration or intense sound.

The assistance rendered by various staff members of this Institute, especially Drs. B. G. King, R. A. Utterback, and A. L. Finkle, in carrying out the experimental work, is greatly appreciated.

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# CARBAMATE CONDUCTION BLOCK IN FROG NERVE FIBERS

FREDERICK CRESCITELLI<sup>1</sup>

*From the Department of Zoology, University of California*

LOS ANGELES, CALIFORNIA

IN A previous communication (1) evidence was given for the appearance of a small, but significant increment in resting potential in a region of living frog sciatic nerve treated with any one of a series of four homologous carbamates. In the concentrations which elicited this positivity the carbamates also produced a readily reversible conduction block in the *A*, *B* and *C* fibers. It is the object of this report to describe certain features in the action of these narcotics in producing this conduction block.

## EXPERIMENTAL PROCEDURE

The procedure has already been described in some detail (1). The carbamates (ethyl-, n-propyl-, n-butyl- and n-amyl-carbamate) were dissolved in phosphate-Ringer's solution at a *pH* of 7.3 and applied to a 25 mm. segment (*segment A*) of isolated frog sciatic nerve. The central sciatic stump was stimulated at a rate of 6 to 12 shocks per minute by means of an electronic stimulator. The monophasic action potentials, recorded oscillographically, were led off from the nerve distal to *segment A*. Conduction block in *segment A* was therefore indicated by a gradual decrease in the spike height. The shock strength and sweep were adjusted differently in different experiments so as to study the impulses in the *A* fibers alone, the *A* and *B* fibers or the *B* and *C* fibers. The experiments were performed at the constant temperature of 25°C. All other conditions were as previously described (1). In this investigation 41 nerves were employed. Of these, 38 were from the bullfrog (*Rana catesbiana*) and three from the grass frog (*Rana pipiens*).

## RESULTS

### *Effect of Previous Treatment*

When any one of the carbamates was added to *segment A* in place of Ringer's solution there occurred a gradual reduction in height of the *A* (fig. 6), the *B* (fig. 3 and 5) and the *C* spikes (fig. 5). A plot of the spike amplitude (in percentage of the original height) against time gave curves (figs. 1, 2 and 4) which describe the course of block well enough for purposes of this report. Such curves permitted the selection of some arbitrary point, e.g. the time for reduction of the spike to the 50 per cent level, which then served as a descriptive time characteristic for the block action. When a nerve was tested repeatedly during the course of 15 to 20 hours with the same concentration of any one of the carbamates the time course of block for any one group of fibers varied from test to test. One important factor in this variability was

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Received for publication September 3, 1948.

<sup>1</sup> Aided by research grants from the Board of Research, University of California. Mr. Philip B. Hollander and Mr. Herman H. Wieder gave valuable technical services during the experimental phases of this work.

TABLE I. THREE-TEST EXPERIMENT ON 12 NERVES

A NERVE	B TEST	C DRUG	D MOLARTIV	E TIME OF TEST, MIN.	F MINUTES TO 50% A HEIGHT	G ½	H ½
11	1	E	0.210		14.0	2.80	1.17
	2			15	5.0		
	3			80	12.0		
13	1	P	0.070		10.2	1.23	0.91
	2			15	8.3		
	3			85	11.2		
15	1	A	0.007		6.0	1.37	0.63
	2			15	4.4		
	3			70	9.6		
16	1	B	0.025		9.0	1.22	1.00
	2			15	7.4		
	3			80	9.0		
17	1	A	0.008		14.8	1.68	1.12
	2			15	8.8		
	3			57	13.2		
18	1	B	0.020		15.1	1.40	1.14
	2			15	10.8		
	3			57	13.2		
19	1	P	0.080		15.0	1.46	0.86
	2			15	10.3		
	3			60	17.4		
20	1	E	0.210		19.8	1.56	0.86
	2			15	12.7		
	3			70	23.0		
68	1	A	0.007		30.0	2.59	0.93
	2			20	11.6		
	3			60	32.3		
70	1	A	0.007		13.6	1.55	0.79
	2			15	8.8		
	3			60	17.2		
71	1	P	0.060		8.4	1.15	0.74
	2			6	7.3		
	3			60	11.3		
72	1	P	0.070		8.4	1.56	1.18
	2			8	5.4		
	3			72	7.1		
Means.....						1.63	0.04

The carbamates are indicated as E (ethyl), P (n-propyl), B (n-butyl), A (n-amyl). In *column E* are indicated the times after washing out the previous carbamate that the indicated tests were made. The ratios of the times to 50 per cent reduction of the A spike of the test 1 to test 2 (*column G*) and the test 1 to test 3 (*column H*) figures are given.

the occurrence of a residual sensitivity in the nerve which remained after exposure to carbamate. A typical illustration of this effect for the *A* group of fibers is given in figure 1A. Curve 1 shows the block action following the initial treatment with carbamate; in this case amyl carbamate, 0.008M. Recovery of the *A* spike required about 6 minutes after removing the carbamate and washing out segment *A* with Ringer's solution (fig. 1B). A second and similar test was then repeated on the same nerve at 15 minutes after removal of the carbamate of the first experiment. Block (curve 2) developed earlier, although recovery was much the same as with the first experiment. A third repetition of the experiment (curve 3) at 57 minutes after removal of the carbamate of the second test demonstrated that the time course of block was approximately similar to that of the initial experiment. A summary of the data (table 1) on 12 nerves offers statistical evidence that the course of block in the *A* fibers was significantly faster when the nerves were tested 15 minutes or less after removal of the carbamate of the first test than if the nerves were tested an hour or

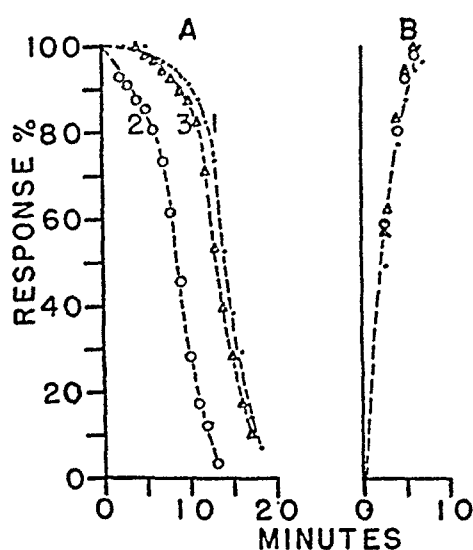


Fig. 1. TIME COURSE of block (A) and recovery (B) of the *A* fibers following the addition and removal of amyl carbamate (0.008M). Data for curve 1 (points) were obtained after the initial addition of drug. Curve 2 (circles) shows the results of the second addition of drug at the 15-min. point in the recovery from *exper. 1*. Curve 3 (triangles) was obtained at the 57-min. recovery point of *exper. 2*.

longer after the removal of the drug of the second test. Thus in a comparison of the ratios for the 50 per cent block time (table 1), the algebraic mean of the test 1 to test 2 ratio was 1.63, significantly greater than unity, whereas the corresponding mean for the test 1 to test 3 ratio was 0.94, not significantly different from unity. The occurrence of a heightened sensitivity which persisted for a time after removal of the carbamate and then disappeared may be related to the residual positivity which was previously shown (1) to outlast the conduction block. It is not known whether these residual effects represent true delays in recovery of those physiological processes which were affected by the carbamate molecule or whether they represent the action of residual carbamate which was only slowly removed after washing.

Most of the experiments to demonstrate the residual sensitivity were carried out with the *A* spikes as indices of response. A few tests made on the *B* fibers indicated that the same phenomenon also appeared with this group. No examination was made of the *C* fibers for residual sensitivity.

### Relative Activity of the Different Carbamates

The alkyl carbamates are known to be surface active compounds whose surface activity increases as the alkyl chain is lengthened. If conduction block involved an adsorption of the narcotic at a surface, the effectiveness of these compounds in producing block should increase in going from the ethyl to the amyl ester. This increase was actually observed. The time course of block of the *A* fibers with different concentrations of any one carbamate varied in the manner indicated by the curves of figure 2A, obtained with *n*-butyl carbamate. From these and similar curves it was

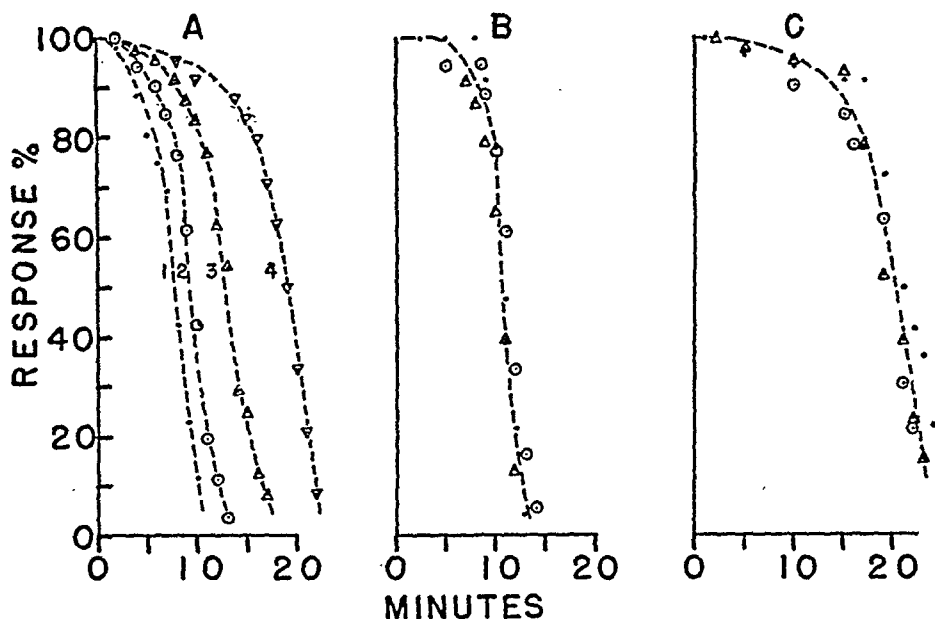


Fig. 2. SERIES A. Time course of block in the *A* fibers with butyl carbamate of concentrations 0.025M(1), 0.023M(2), 0.020M(3) and 0.018M(4). At least 1 hr. was allowed for recovery between each experiment. SERIES B. Approximately matched responses of a nerve to 3 carbamates: propyl carbamate (0.07M), shown as *points*; butyl carbamate (0.018M), as *circles*; and amyl carbamate (0.0065M), as *triangles*. Liquid-air surface tensions of these 3 solutions, as interpolated from curves of surface tension versus concentration (measurements with a tensiometer) were 61.7, 62.8 and 57.5 dynes/cm., respectively. SERIES C. Approximately matched responses of a nerve with: ethyl carbamate (0.21M), as *triangles*; propyl carbamate (0.07M), as *points*; and butyl carbamate (0.018M), as *circles*. The liquid-air surface tensions of these 3 solutions were 62.8, 61.7 and 62.8 dynes/cm., respectively.

then possible to compare the concentrations of the different carbamates which gave approximately the same block curve. The relative effectiveness of the propyl, butyl and amyl esters are compared in figure 2B while a similar comparison for the ethyl, propyl and butyl compounds is shown in figure 2C. The carbamate concentrations which gave these approximately matched pharmacological responses also reduced the surface tension of Ringer's solution to about the same degree (fig. 2 legend). The relative effectiveness of these compounds in blocking conduction was thus roughly of the same order as their ability to reduce surface tension.

### Differential Sensitivity of the Fiber Groups

A. *B fibers*. Much evidence was obtained to show that the different fiber groups of the bullfrog sciatic-peroneal nerve responded quite differently to these narcotics.

In this action all four carbamates behaved similarly. The most sensitive fibers proved to be those of the *B* group. These ceased conducting before either the *A* or the *C* fibers. A typical example of the differentiation between the *B* and *A* groups is shown in figure 3. In a number of experiments the *B* spike was completely abolished while the *A* spike was still at its original level, indicating that all the *B* fibers were blocked before any significant number of *A* fibers had ceased conducting (fig. 4). In recovery, the *B* fibers, as a group, lagged significantly behind the *A* fibers (fig. 4). The data from 11 experiments show that on the average the time to reduce the spike to half its original value is about three times as great for the *A* as for the *B* group of fibers (table 2).

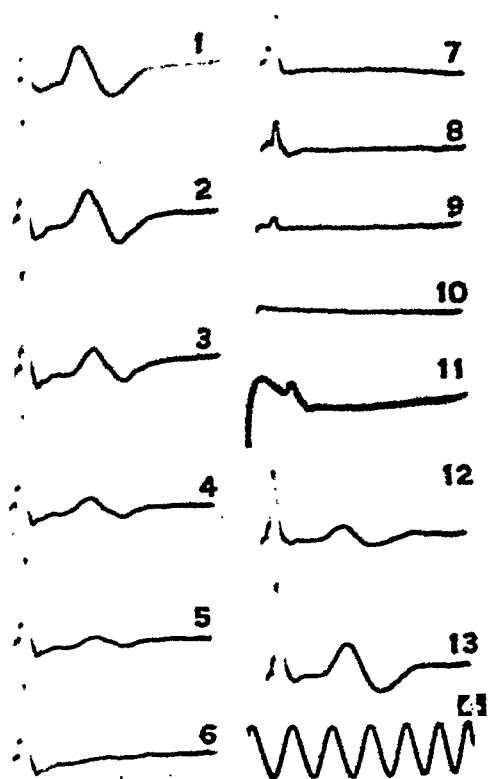


Fig. 3. DIFFERENTIAL CONDUCTION BLOCK of the *A* and *B* fibers by propyl carbamate (0.07M). Record 1 is the Ringer's sol. control. The others were photographed 4'(2), 7'(3), 8'(4), 9'(5), 10'(6), 13'(7), 18'(8), 24'(9) and 30'(10) after adding the drug. Recovery is shown at 4'(12) and 7'(13) after washing out the drug. The 60 c.p.s. time line is in record 14. Record 11 indicates that some of the *C* fibers were still conducting after both *A* and *B* groups were all blocked.

A comparison of the *B* and *C* fibers is made in figure 5. In this experiment complete block in the *B* fibers occurred with no significant reduction in the *C*<sub>2</sub> action. Two experiments in which the *C* spike was sufficiently developed to permit accurate measurements are included in the data of table 2.

B. *A* fibers. As is well known (2) the *A* fibers of the bullfrog sciatic nerve consist of several groups which appear as discrete peaks in the compound *A* spike. It is of interest to inquire whether these components of the *A* group can be differentiated in any way through the action of the carbamate molecule. Generalization is made difficult and uncertain because of the varying behavior of different nerves or of even the same nerve at different examinations. Ignoring these variations, the results as a whole indicate some differentiation in the *A* fibers. The most prominent feature

of this differentiation was that the smallest (gamma) fibers were most resistant. A typical experiment (fig. 6) illustrates the persistence of some gamma fibers after all the alpha and beta fibers were blocked. A similar differentiation was obtained in eight nerves in which the gamma spike was distinct enough to be recognizable.

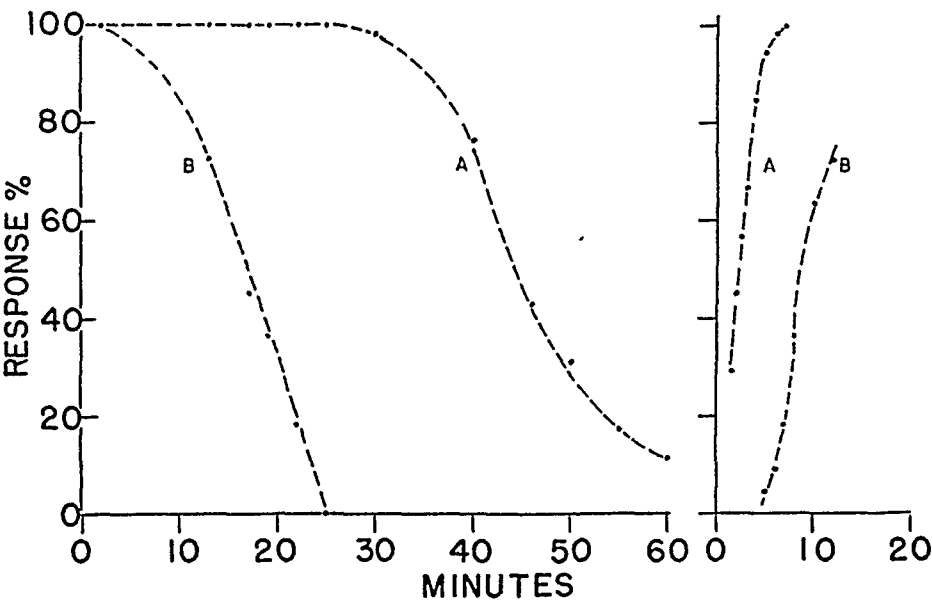


Fig. 4. TIME COURSE of block and recovery in *A* and *B* fibers following the addition and removal of propyl carbamate (0.07M).

TABLE 2. DIFFERENTIAL SENSITIVITY OF FROG NERVE FIBERS

NERVE	DRUG	MOLARITY	MINUTES TO 50% BLOCK			RATIO OF A TO B BLOCK TIME
			A	B	C	
26	P	0.06	66.0	18.5		3.6
27	B	0.02	25.0	6.0		4.2
28	P	0.08	19.2	10.0		1.9
28	A	0.007	29.5	9.0		3.3
29	B	0.023	18.4	9.0		2.0
31	P	0.10	8.2	5.0		1.6
35	A	0.008		4.6	15.5	
35	A	0.008	14.3	6.5		2.2
68	A	0.007	30.0	4.1		7.3
68	A	0.007	32.3	7.1		4.5
73	P	0.07		9.3	52.7	
73	P	0.07	12.8	7.9		1.6
76	P	0.07	45.0	16.9		2.7
Mean.....						3.2

C. *C fibers*. It has already been indicated that the *C* fibers as a group blocked less rapidly than did the *B* fibers. A simultaneous comparison of the *C* and *A* fibers was not made because of the large artifact introduced into the *A* spike as a result of the increased shock strength and increased amplification required to bring out the *C* spikes. It was possible to show, however, in a number of experiments that when



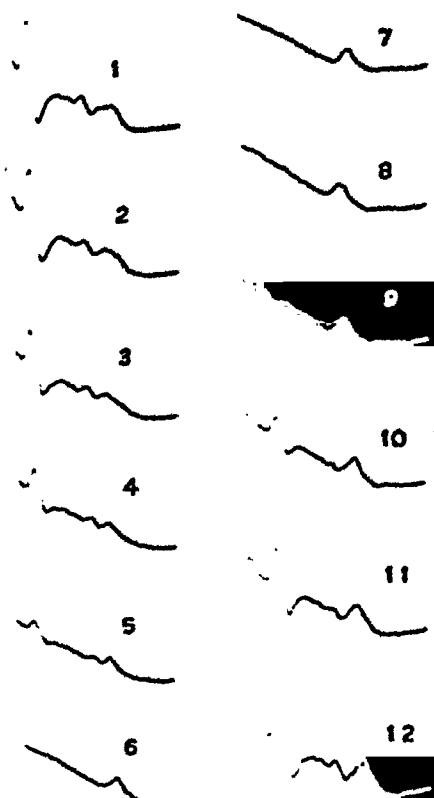


Fig. 5. DIFFERENTIAL CONDUCTION BLOCK in the B and C groups of fibers by propyl carbamate (0.07M). Record 1 is the Ringer's sol. control. The drug action is shown at 2'(2), 4'(3), 6'(4), 8'(5), 12'(6), and 45'(7). Recovery is indicated at 1.5'(8), 2'(9), 3'(10), 6'(11) and 10'(12). Note persistence of the C<sub>2</sub> action long after both B and C<sub>1</sub> potentials had been abolished.

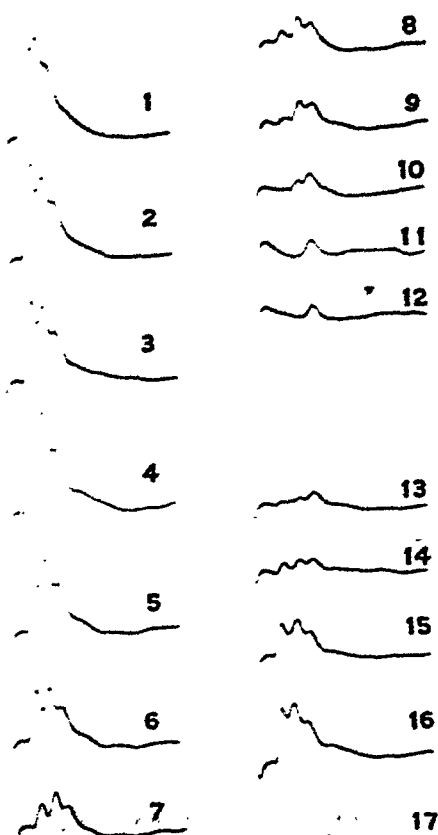


Fig. 6. CONDUCTION BLOCK in the A group of fibers. Record 1 shows the A spikes with segment A in Ringer's sol. The differential block by butyl carbamate (0.017M) is indicated in records 2-12, taken 14'(2), 20'(3), 30'(4), 34'(5), 38'(6), 43'(7), 48'(8), 51'(9), 56'(10), 68'(11) and 72'(12) after adding the drug. Amplitudes are not directly comparable in all records as the amplifier gain was increased between records 3 and 4 and decreased again between records 12 and 13, indicated by change in size of the shock artifact. Recovery, incompletely indicated by records 13-16, occurred after washing out segment A. These records were made at 1.3'(13), 2.3'(14), 5'(15), and 8'(16) after washing. Record 17 is of the 1000 c.p.s. time line.

both *B* and *A* fibers had ceased conducting, an appreciable proportion of the *C* spike was still present. This is illustrated by the experiment in figure 3. Records 2 to 10 show the disappearance of first the *B*, then the *A* spike, so that record 10 shows no evidence of either *A* or *B* fiber activity. Record 11 was photographed one minute after record 10. It pictures the *C* action resulting from the increase in shock strength. The *C* spike in this record was about 60 per cent of the original *C* height at the beginning of this series. This experiment demonstrates the relative insensitivity to carbamate of some *C* fibers.

The *C* spike of the bullfrog sciatic-peroneal nerve was composed typically of two components. In these experiments indications were obtained that  $C_1$  and  $C_2$  were also capable of being differentiated by the carbamates (fig. 5). The evidence at hand on this point is neither abundant enough nor certain enough to permit any further statements at this time.

#### DISCUSSION

Essentially, this report describes certain phenomena observed during conduction block in frog nerves treated with relatively dilute concentrations of the carbamates. Neither here nor in the existing literature is there sufficient information to permit a conclusion regarding the mechanism of action of these compounds in producing conduction block. Nevertheless, certain statements may be made which assist in integrating some of the published data. In a previous report (1) reasons were presented for the concept that the increase in resting potential following treatment of the nerve with these compounds may be associated with a decrease in permeability. Evidence already exists suggesting that carbamates in the proper concentrations are able to decrease the permeability of a number of cells (3-6). There is good agreement between the figures for the concentrations which have been reported to decrease permeability (5, 6) and which here have been shown to produce a readily reversible conduction block.

At present a student of the nerve impulse is presented with two working hypotheses: 1) that conduction is associated with a transient increase in permeability (7, 8) and 2) that conduction block, at least with the carbamates, is associated with a decrease in permeability. It is of course a key problem and one which is the cause of much controversy to decide whether the passage or release of some specific substance is the primary event in conduction and if so to reveal the nature of this substance. Narcotics, such as the carbamates, might then be visualized as causing block by interfering with the release of or preventing the passage of such an essential substance. The immediate problem appears to be whether substances like the carbamates may interfere with the release or transfer of  $K^+$ . Hodgkin and Huxley (8) recently reported that the increased conductance following activity in isolated axons of *Carcinus maenas* was associated with the release of a substance having effects on conductance similar to the effects of  $K^+$ . Shanes (9) has also considered this problem in relation to the mode of action of cocaine. He concluded that the permeability of muscle and nerve to  $K^+$  is reduced by this narcotic. All these studies open several paths for future investigation.

The differential fiber sensitivity of frog nerves to carbamates resembles the

differential effects of hypoxia or anoxia. It has been shown (10, 11) that the *B* fibers in mammalian nerves are most quickly blocked by lack of oxygen while the *C* fibers are the most resistant. A similar behavior of bullfrog nerves has recently been reported (12). The similarity in response of nerve to anoxia and to the carbamates may be simply coincidental, but it is of interest to point out that the carbamates in the same range of concentrations as employed in this work are able to depress the oxygen consumption of many living cells (13-16).

The differential blocking action of the carbamates does not resemble the action of other narcotics. Cocaine, for example, has been reported to block the *C* fiber group before the *A* fibers (17). Procaine appears to have a similar action (18). Moreover, in contrast to the carbamate type block, cocaine is known to affect frog and mammalian *A* fibers differentially, with the smaller fibers blocking first (17, 19). Ether and nembutal have been reported (20) to act differentially on the fibers of the turtle vagus nerve with the *C*, *B* and *A* fibers blocking in the order named. In an interpretation of the selective action of cocaine on the *A* fibers, Gasser and Erlanger (19) suggested that either the thinner myelin sheath or the greater surface per volume ratio might be responsible for the greater susceptibility of the smaller fibers. It is obvious that neither of these suggestions is adequate to explain the carbamate type of differentiation. Lorente de N6 (12) has also emphasized the point that the thickness of the myelin sheath appears to have little to do with the action of a number of substances. The work of the Japanese investigators (21) showing that the nodes of Ranvier are regions of particular sensitivity to narcotics may render irrelevant any consideration of the myelin sheath as a barrier to the penetration of substances.

#### SUMMARY

Certain features in the blocking action of ethyl-, n-propyl-, n-butyl- and n-amyl carbamate are described. These esters were able to block selectively the different fibers of the bullfrog sciatic nerve. The *B* fibers as a group were most readily affected. The time to reduce the spike height to 50 per cent of its original level was about three times as great for the *A* fibers as for the *B* fibers. Among the *A* fibers, the gamma fibers were typically most resistant. There was some difference in behavior of the different *C* fibers but a large proportion of them were able to conduct after all *A* and *B* fibers had been blocked. The relative effectiveness of the four different carbamates in blocking the *A* fibers was found to be approximately the same as their relative effectiveness in reducing surface tension. Evidence for the existence of a period of heightened susceptibility to block by carbamate was noted following the washing out of a previously added carbamate and the complete recovery of ability to conduct.

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# SHOCK DUE TO HEAD INJURY IN THE FROG

LOUIS MOREAU<sup>1</sup>

*From the Zoological Laboratory of the University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

**D**EATH from head injury in the absence of demonstrable histo-pathological changes has long been an enigma (1-3). Evidence has been reported (3) for the rôle of a reversible 'molecular reaction' in the concussion state. In this report the concussion state was shown to be independent of vascular spasm, hemorrhages, stasis of the brain, fat embolism or any other detectable histopathological lesions. Earlier work in this laboratory (4) has shown that in electrical injury to the brain there is released into the blood stream a toxic factor that has thromboplastic properties. There are other suggestions of a possible connection between the phenomenon of toxicity and thromboplastic properties. The placental toxin, which is considered to play an etiological rôle in eclampsia, has been shown by Schneider to be thromboplastin (5). Moreover, thromboplastin has been shown to be involved in producing some of the toxic effects of muscle ischemia (6). Most of the literature on the relation of injury to protoplasmic clotting has been reviewed already by Heilbrunn (7-9).

The question arises as to whether head injury could be followed by the release of thromboplastic substances into the blood. If this is true, head injury should be followed by disturbances of the blood coagulation. The brain is an especially rich source of thromboplastic material (10, 11), and brain extracts have been shown to be highly toxic (12, 13). Thus, it is possible that head injury can produce toxic effects not immediately due to nerve impulses. Accordingly, it was attempted to determine whether visible toxic effects would follow head injury in animals whose spinal cords had been severed; and, also, to record the associated changes in the blood-clotting time.

## METHODS

Frogs, *Rana pipiens*, weighing 18 to 23 grams and of both sexes were chosen as the experimental animal. In all, 422 animals (including 164 controls) were used. Earlier experiments were performed without any special equipment. Head trauma was produced by grasping the frog and striking its head on the edge of a table. Afterwards, trauma was given with a light hammer, a stiff rubber tube or by crushing the head with narrow pliers. The best results were obtained with a traumatizing device. This device consisted of an arm powered by a ratchet wheel so that it could be brought down repeatedly on the head of the animal with uniform force. Each turn of the wheel caused the arm to fall four times.

In order to eliminate the effect of nerve impulses and muscular fatigue attendant

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Received for publication August 9, 1948.

<sup>1</sup> National Institute of Health Fellow.

upon the convulsive effects of head trauma, the spinal cord was severed by means of a small knife made of flattened iron wire approximately 2 mm. wide. A small transverse incision was made over the foramen magnum with a scalpel. Then the knife was inserted straight across the spinal cord and moved sidewise two or three times. The main difficulty with this procedure lies in the excessive bleeding that may occur upon the accidental rupture of the spinal vessels. All animals that showed excessive bleeding were eliminated from the experiments, for the reason that excessive bleeding leads to hemodilution, which results in prolongation of the clotting time.

The clotting time of the blood was determined by the capillary method. Samples were obtained from the exposed heart, by puncture with a capillary tube. The details and precautions of this method are given in the earlier paper (4).

#### EXPERIMENTS

*General Effects of Head Injury upon the Frog with Transected Spinal Cord.* In preliminary experiments 55 frogs were given head trauma by relatively inexact methods: striking on the table, using the light hammer, striking with a stiff rubber tube and crushing with pliers. Of these 55 animals, 14 went into irreversible depression in 30 to 60 minutes; 14, in 6 to 8 hours; 10, in 8 to 10 hours and 12, in 12 to 24 hours.

It was noted that most of the animals with the longer survival time had received greater apparent injury: the skull had been crushed by the traumatic procedure. Most of the animals with shorter survival times had received less apparent injury. From these results it appeared that if a toxic factor was produced it did not always gain access to the general circulation. Occlusion of blood vessels resulting from the excessive bone and tissue crushing was thought to be involved.

The traumatizing machine was then made for the purpose of minimizing the crushing effects of trauma. With the arm adjusted so that the force of each tap was approximately 500 grams, 10 frogs were traumatized with 200 taps. When this method is properly administered in frogs (after transection of the spinal cord) it is not attended by any noticeable convulsive effects. The hind legs remain in a natural position. After the trauma there is a brief period (approximately 30-45 seconds) in which the hind legs are not responsive to pricking with a needle; but after this, they become normally responsive, although the frog does not move about on its own initiative. None of the 10 frogs traumatized with the 500 gram force was seen to show any signs of depression for at least 8 hours.

The arm was readjusted so that the force of each tap would be approximately 725 grams. Of 12 frogs given 200 taps, 10 were in irreversible depression within 35 minutes. The remaining 2 succumbed in 4 hours. In frogs weighing 18 grams or more, this method of traumatizing produces no bone-crushing effects. This method was adopted for all the remaining experiments.

The observable characteristics of depression due to head trauma were as follows: for about 30 to 45 seconds following trauma the hind legs were flaccid and unresponsive to prodding. After this they recovered normal reactivity for a variable period, but most commonly for 30 to 60 minutes; then a generalized depression followed.

This depression was characterized by a gradually decreasing responsiveness of the front and hind legs, accompanied by sluggishness of the corneal reflex. The corneal reflex sometimes outlived the responsiveness of the legs by 15 to 20 minutes, but sometimes was entirely lost before the legs became altogether unresponsive to mechanical stimuli. Usually about 15 to 20 minutes were required for the complete loss of reactivity of the legs. The heartbeat continued with gradually decreasing strength, force and rate for  $2\frac{1}{2}$  to 4 hours after the onset of depression.

*Depressant Effect of Transection of Spinal Cord.* Spinal transection itself, without trauma to the head, produces signs of irreversible depression after a few hours. Seventeen frogs were transected and placed in a pan containing a small amount of water. The survival time of these animals ranged from 1 to 20 hours (av. = 4.3 hours).

Another experiment was done in which 20 frogs were transected, but 10 of these were given head trauma, in addition. The animals were placed in similar containers and observed. The results are shown in table 1, which shows that head trauma shortened the average survival time by 75 per cent.

TABLE 1. COMPARISON OF THE SURVIVAL TIME OF FROGS AFTER TRANSECTION OF THE SPINAL CORD WITH AND WITHOUT HEAD TRAUMA

NO.	1	2	3	4	5	6	7	8	9	10	AVERAGE
Survival time; (hr:min.)											
Transection and trauma.....	0:05	0:10	0:30	0:30	0:30	0:25	1:20	1:40	2:30	2:50	1:03
Transection.....	0:45	1:40	2:40	3:20	3:20	5:00	5:30	6:00	6:00	7:30	4:04

*Effects of Head Trauma on Blood Coagulability.* A preliminary study made by Mr. J. T. Hicks, in this laboratory, showed that clotting time increased after head injury. The average clotting time in 128 untraumatized frogs was 3.6 minutes; in a separate group of 128 frogs, the clotting time 20 minutes after trauma was found to be 12.1 minutes. In these experiments the spinal cord was not transected. Trauma was given by striking the frogs on the edge of a table. The results of this experiment differ somewhat from those of other experiments and will be commented on later.

Using the traumatizing machine after cord transection, the clotting time was found to be decreased immediately after trauma. Sixteen animals with transected cord were used as controls, and had an average clotting time of 3.2 minutes. In 10 animals with both cord transection and head trauma the average clotting time, immediately after trauma, was 1.0 minute.

Another experiment on 5 frogs in which the clotting time was determined in each frog immediately before and after trauma also indicated a decrease, though not as marked, as in the previous experiment. The average clotting time was one minute, 47 seconds before trauma, and one minute, 7 seconds after trauma.

The next experiment consisted of serial determinations in which, for each animal, the clotting time was determined at intervals over a period of time. Eleven test animals and 11 controls were used. The test animals fell into two almost equally divided groups: one with a relatively high initial clotting time and the other with a relatively low initial clotting time. This difference in clotting times was

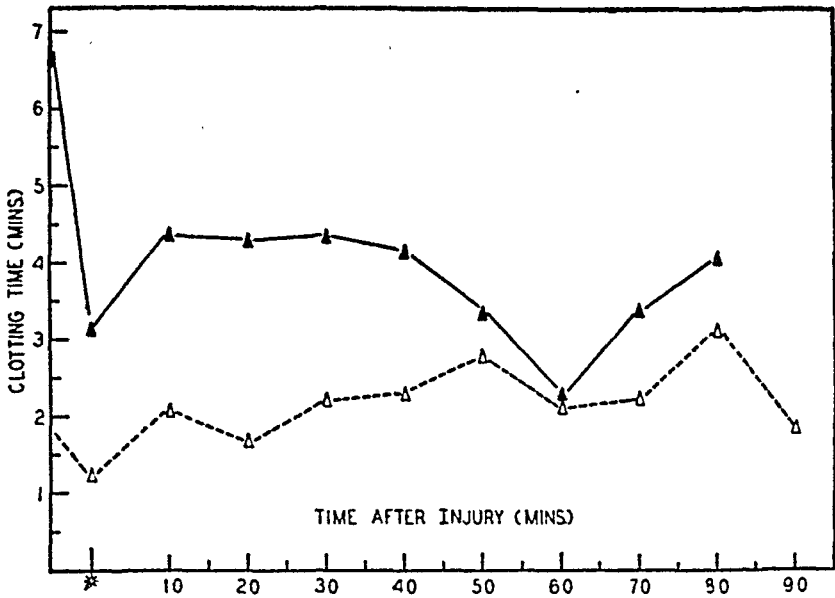


Fig. 1. EFFECT OF HEAD INJURY ON BLOOD COAGULABILITY OF FROG. *Upper curve*, average values obtained for 6 frogs. It was not possible to obtain blood samples from one of the animals after 40 min., and from 2 other animals after 50 min. After these points the curve represents values obtained from the 3 remaining animals. *Lower curve*, averages obtained from 5 animals. Sampling became impossible for 2 of the animals after 50 min.

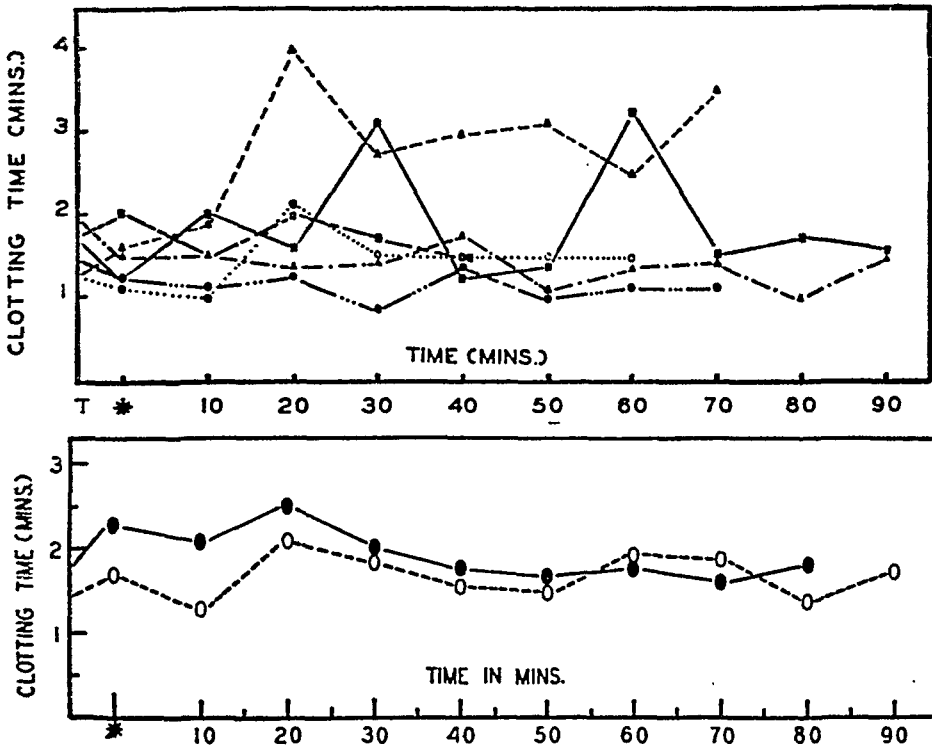


Fig. 2. CONTROLS (*upper*). Changes in blood-clotting time of 6 frogs with transected spinal cord, but no head injury.  
Fig. 3. CONTROLS (*lower*). Changes in blood coagulability in frogs which were not given head injury. *Upper curve* represents those with transection of the spinal cord; *lower curve* represents those which were given no treatment.

encountered in frogs used in studying the effects of electrical injury (4). Possibly the reason for the differences in clotting time in these animals is that the experiments



were done during the summer. The frogs had been transported during the hot weather and some of them might have suffered some degree of heat injury. Nevertheless, whatever the cause of the difference in clotting time it is not considered significant from the point of view of these experiments, since the effects of trauma were substantially similar in both the high and the low groups (fig. 1). In these experiments the clotting time 20 minutes after trauma was not elevated. This differs greatly with the results of the previously mentioned experiment in which the spinal cords of the animals were intact and only one blood sample was taken. In this experiment the clotting time was distinctly elevated at 20 minutes after trauma. However, no definite statement can be made at this time to explain the difference.

Eleven frogs served as controls. In 6 of the controls spinal cord transection was done before determining the clotting times. The results were similar in both groups. An idea of the degree of individual variation can be obtained from figure 2 which shows the curves obtained from the control animals with cord transected. Figure 3 shows the average values obtained from the controls.

#### DISCUSSION

The foregoing experiments have shown that mechanical injury of the head can produce delayed, irreversible depression in the frog. The experiments were planned so as to eliminate factors other than circulatory. No amount of head injury was able to cause death when the injury was such as to crush the brain case and thus to impede the exit of cerebral blood to the general circulation. Therefore, it is most reasonable to deduce that the depression observed was due to the release of some toxic factor from the injured head into the bloodstream.

In the past it has been abundantly shown that both anaphylactic shock and clinical shock are accompanied by prolongation of the blood-clotting time (14, 15). Generally, it has been held that the prolongation of the clotting time, due to an excess of blood heparin, is the manifestation of a mechanism counteracting some increased thrombogenic tendency of the blood. In studies of tourniquet shock by Mylon *et al.* (16) release of the tourniquet was followed by an immediate decrease in the blood-clotting time. This was followed by an increase in clotting time. In the present experiments, there was obtained evidence for the rôle of a thromboplastic substance in shock due to head injury. It was observed that injury was followed by a disturbance of blood coagulation. This disturbance is characterized by an initial decrease in clotting time, followed by greater or lesser increases and decreases.

The present experiments are corroborative of earlier experiments (to which reference has already been made, 4) on the effects of electrical injury. In these earlier experiments shock was accompanied by disturbances of blood coagulation similar to those in head injury. Moreover, in the experiments on electrical injury of the hind legs, the depth of depression could be controlled by the alternate application and removal of ligatures proximal to the injured areas.

The findings recorded in these experiments are in accord with the theory that in injury shock a circulating toxic factor is involved; and that this toxic factor is a substance with thromboplastic properties.

## SUMMARY

Mechanical injury of the head produced profound physiological depression and death in frogs whose spinal cords had been transected. Regardless of the amount of trauma, the toxic effects were absent or decreased when trauma was accompanied by obstruction of the cerebral blood flow due to crushing of the skull. These observations suggest the presence of a circulating toxic factor. Disturbances in the blood-clotting mechanism after injury indicate that the toxic factor is a substance with thromboplastic properties. These results are correlated with the results of previous work favoring the concept that thromboplastic substances may be involved in the pathogenesis of injury shock.

The author wishes to give grateful acknowledgement to Professor L. V. Heilbrunn for his kindly interest and guidance in the conduct of this study.

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# PULMONARY CAPILLARY PRESSURE IN ANIMALS ESTIMATED BY VENOUS AND ARTERIAL CATHETERIZATION

HARPER K. HELLEMS,<sup>1</sup> FLORENCE W. HAYNES, LEWIS DEXTER AND  
THOMAS D. KINNEY

*From the Department of Medicine and Pathology, Peter Bent Brigham Hospital and Harvard  
Medical School*

BOSTON, MASSACHUSETTS

**B**Y MEANS of the technique of venous catheterization of Cournand and Ranges (1), it has been possible to record pressures in the great veins, right auricle, right ventricle and pulmonary artery with suitable recording devices. This study describes a method by which the pressure in the pulmonary capillaries can be estimated by means of the venous catheter.

## METHODS

Dogs anesthetized with nembutal (40 mg/kg. intraperitoneally) were used in the present study. A no. 8 French catheter, 2.5 mm. in diameter, with a hole in the tip, was introduced into the external jugular vein and guided fluoroscopically, as described elsewhere (2), into a branch of the pulmonary artery. It was pushed as far as possible and wedged in a distal ramification so as to obstruct the vessel.

A no. 8 French catheter was introduced by way of the carotid artery into a pulmonary vein on the same side of the lung as the first catheter (3). The carotid artery was exposed and two loops of silk served to lift the artery out of the wound and to occlude blood flow temporarily. A small opening was made through the arterial wall with a sharp no. 14 needle and a no. 8 French catheter was introduced through this hole into the arterial lumen. If a snug fit was obtained, no bleeding occurred and the silk stays could be removed. The catheter was guided fluoroscopically into the ascending aorta by keeping the curved tip to the animal's right. When resistance was encountered at the aortic valve the catheter was introduced through the valve into the left ventricle by gently withdrawing and advancing the catheter until it slipped through the valve during ventricular systole. The tip was then directed posteriorly and to the right and inserted further causing a bend to appear with its convexity to the left and with the tip pointing medially and superiorly in the direction of the mitral valve. It was then advanced through the valve into the left auricle and into one of the pulmonary veins. Here it was wedged so as to obstruct the lumen of the pulmonary vein.

A third catheter, no. 9 French, was introduced through the other jugular vein into a branch of the pulmonary artery of the opposite lung, but was not allowed to obstruct the vessel. This catheter was used for recording pressure in the pulmonary artery and for the injection of embolic material. Pulmonary embolism was produced by injecting through this catheter a one per cent suspension of lycopodium spores at the rate of 3 cc/min. into a local area of the lung distant from the location of the other two catheters. The purpose of this was to raise the pressure in the pulmonary artery, pulmonary capillaries and pulmonary vein (4). Figure 1 is an X-ray of the chest with the three catheters in position. To determine the effect of respiration on the pressures recorded in the ob-

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Received for publication August 3, 1948.

<sup>1</sup> This work was done during the tenure of a Life Insurance Medical Research Fellowship and supported by a grant from the Life Insurance Medical Research Fund.

structed artery and vein, two experiments were performed with controlled respiration after the spinal cord had been pithed.

The position of the catheters was verified by chest X-rays taken during the experiment, and all dogs were autopsied to confirm the location of the catheters and to allow pathological inspection of the cardiac chambers and lungs.

Pressures were recorded with both the optical manometer of Hamilton (5) and a column of saline as is commonly used for measuring venous pressure. The zero point for all pressures was taken 7.5 cm. anterior to the spine with the dog lying on its back.

### RESULTS

The contours of the pressure curves recorded through the catheters occluding the pulmonary artery and vein were similar. As can be seen in figure 2, no pulse wave



Fig. 1. ANTERO-POSTERIOR ROENTGENOGRAM of chest showing location of catheters. *a.* Obstructing right lower lobar branch of pulmonary vein. *b.* Obstructing right lower lobar branch of pulmonary artery. *c.* Free in left lower lobar branch of pulmonary artery.

was obtained but there was considerable respiratory variation in the recorded pressure, the pressure being lower in inspiration than in expiration by an average of 7 mm. of Hg (tables 1 and 2). The small waves in the tracing were artifacts, presumably resulting from the bending of the catheter during ventricular systole.

Mean pressures recorded through the catheter wedged into the pulmonary artery in 24 dogs varied between 2 and 8 mm. Hg with an arithmetical mean of 4 mm. Hg (tables 1 and 2). In 6 dogs in which the mean pressure was recorded simultaneously through the catheter obstructing the pulmonary vein the pressure was uniformly higher, on repeated determinations in each experiment, by an average of 4 mm. of Hg (table 1). This same relationship was maintained in both inspiration and expiration with the exception of *dog 82* in which it was reversed in both phases of respiration. However, in this animal the mean pressure through the catheter obstructing the pul-

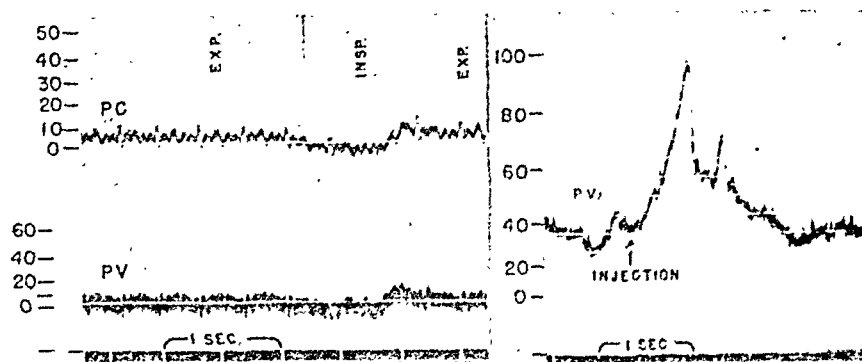


Fig. 2 (left). SIMULTANEOUS RECORDING, with Hamilton manometer, of pressure in blocked pulmonary artery (P.C.) and blocked pulmonary vein (P.V.). Note that contours of curves are identical.

Fig. 3 (right). RECORDING OF PRESSURE in obstructed pulmonary vein (P.V.) showing rapid increase in pressure resulting from injection of 1 cc. of saline through the opposing catheter wedged into the pulmonary artery.

TABLE 1. VARIATIONS IN PRESSURE IN AN OBSTRUCTED PULMONARY ARTERY AND VEIN IN 6 NORMAL DOGS AND IN 4 DOGS WITH PULMONARY HYPERTENSION AS A RESULT OF PULMONARY EMBOLISM

All values in mm. Hg

Exp. No.	PRESSURES WITH CATHETER IN										EST. PULMONARY CAPILLARY PRESSURE WITH FREE FLOW	
	Pulmonary artery		Pulmonary end artery				Pumonary end vein				Hamilton manometer mean	Saline manometer mean
	Hamilton ma- nometer		Hamilton manom- eter			Saline manom- eter mean	Hamilton manom- eter			Saline manom- eter mean		
	Syst.	Diast.	In- spir.	Ex- pir.	Mean		In- spir.	Ex- pir.	Mean			
67	45	20	5	12	8	5	8	13	10	12	9	8.5
68	26	10	1	10	6	6	11	18	14	14	10	10
70	26	18	2	10	5	6	6	11	9	9	7	7.5
73	26	9	4	12	7		10	14	12	16	9.5	
82			4	9	5	8	2	8	6	9	5.5	8.5
83			2	8	6	6	6	15	11	11	8.5	8.5
Average....	31	14	3	10	6	6	6	13	10	12	8.3	8.6

After onset of pulmonary hypertension produced by injection of lycopodium spores into pulmonary artery of opposite lung

67	80	48	4	14	10	14	8	17	15	22	12.5	18
68	64	35	8	18	13	11	14	30	22	27	17.5	19
70	88	38	11	18	14	16	14	21	19	20	16.5	18
82	70	30	10	21	17	19	20	32	26	26	21.5	22.5
Average....	76	38	8	18	14	15	14	25	21	24	17	19.4

monary vein was one mm. of Hg higher than through that obstructing the pulmonary artery. The mean pressures recorded by the saline manometer were in close agreement with the mean pressures obtained with the Hamilton manometer.

In one experiment, dog 82, catheters were wedged fortuitously into the pul-

monary artery and vein in the same part of the lung. Fluoroscopically, they appeared in juxtaposition when viewed in the right anterior oblique position. A rapid injection of 1 cc. of saline through either catheter produced a sharp rise of over 50 mm. Hg of pressure in the catheter wedged into the vessel on the opposite side of the capillary bed (fig. 3), indicating not only a free communication between the two but also the relatively small volume of the intervening capillary bed.

When the pressure in the pulmonary artery was raised by the injection of lyco-podium spores through a third catheter located in the artery (4) of the opposite lung,

TABLE 2. VARIATIONS IN PRESSURE IN PULMONARY ARTERY AND IN OBSTRUCTED PULMONARY ARTERY IN 18 NORMAL DOGS UNDER NEMBUTAL ANESTHESIA  
*Values in mm. Hg*

EXP. NO.	PRESSURES WITH CATHETER IN					
	Pulmonary artery		Pulmonary end artery			
	Hamilton manometer		Hamilton manometer			Saline manometer mean
	Syst.	Diast.	Inspir.	Expir.	Mean	
86	21	7	2	4	3	3
88	30	8	0	5	3	2
89	28	8	-5	9	2	2
90	17	8	-1	9	4	3
91	30	12	-2	9	4	5
92	25	6	1	5	3	3
93	35	12	0	13	8	9
94	20	8	0	9	5	1
95	31	14	3	11	6	6
96	30	3	0	6	4	3
97	20	7	0	6	4	7
99	28	9	0	6	4	4
100	21	6	-5	7	2	2
101	23	3	0	4	3	3
102	32	12	0	8	5	7
103	—	—	-2	6	2	3
104	24	9	2	4	3	6
105	22	8	-2	5	2	2
Average...	26	8	0	7	4	4

pressures recorded through the catheters wedged into the pulmonary artery and vein eventually became elevated but maintained their same general relationship, i.e. were higher on the pulmonary venous than on the pulmonary arterial side (table 1).

To determine the effect of respiration on the two pressures, two experiments were done in which the animal was maintained on artificial respiration after the spinal cord was pithed. Under artificial respiration, pressures recorded with the catheter in the pulmonary vein were higher than those recorded in the pulmonary artery. When the artificial respiration was stopped for one minute, both pressures fell about 2 cm. of water (fig. 4). The pressure through the catheter obstructing the pulmonary vein, however, continued to be higher than that recorded through the catheter obstructing

the pulmonary artery. Immediately after the heart failed to show electrical activity by electrocardiogram and therefore the flow of blood had ceased, the pressures measured on the two sides of the capillary bed became equal (if corrected so that the tips of the catheters were at the same hydrostatic level). The induction of artificial respiration in this dead animal caused a slight increase in both pressures, but this rise in pressure was of the same magnitude in each.

In the 6 animals in which the catheter was passed through the left side of the heart, 4 showed damage consisting of small endocardial hemorrhagic areas on the mitral and aortic valves and in one animal there was a subendocardial hemorrhage in the wall of the left ventricle.

The catheter which obstructed the pulmonary artery for one to four hours produced no changes suggestive of infarction of the lungs.

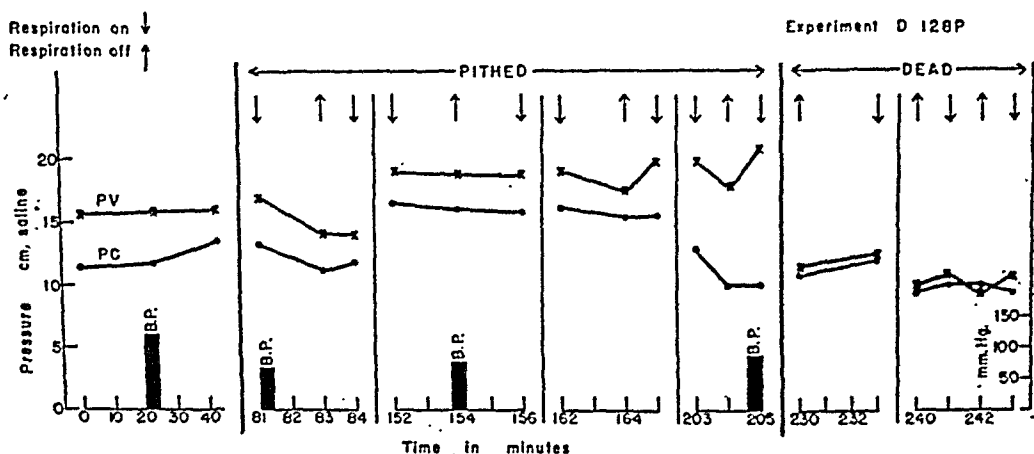


Fig. 4. EFFECT of artificial respiration on pressures recorded through catheters obstructing pulmonary artery (P.C.) and pulmonary vein (P.V.).

Note 1) that pressure on the venous side is almost uniformly higher than that on the arterial side; 2) that when blood flow ceases (death), the pressures fall to essentially the same level indicating that (1) above is an artifact due to blood flow; and 3) that when respiration is induced, both pressures increase slightly (pumping action of the lung).

#### DISCUSSION

Anatomical studies of the vessels of the lung by histological and injection techniques have indicated the following features which are pertinent to the present study. The pulmonary artery undergoes a series of subdivisions finally ending in a rich plexus of freely anastomosing capillaries. Bronchial arteries likewise subdivide and end in capillaries which anastomose freely with the capillaries of the pulmonary artery. In a review of the literature and from his own observations, Miller (6) concluded that there are normally no anastomoses between the bronchial and pulmonary arteries proximal to the pulmonary capillary bed. Bruner and Schmidt (7) have also arrived at the same conclusion. Physiologically the same has been demonstrated (8) in that blood, fully saturated with oxygen, may be withdrawn through a catheter wedged into a branch of the pulmonary artery of patients thus indicating that the catheter was in direct communication with the capillary bed and that any significant number of precapillary anastomoses between different branches of the pulmonary artery did not exist. If such were present, this blood would be unsaturated. In patients with

arterial oxygen unsaturation, blood withdrawn in a similar fashion has been shown to be fully saturated with oxygen, indicating the absence of any significant precapillary anastomoses between systemic (bronchial and intercostal) arteries and pulmonary artery (9).

Anatomically there are no valves in the pulmonary vessels (6). Dyes injected into a pulmonary vein in a retrograde direction flow into the pulmonary capillaries and then into the pulmonary and bronchial arteries (10). These observations are confirmed in the present study in which catheters were wedged into a pulmonary artery and vein in close approximation one to the other. A quick injection of only 1 cc. of saline into either catheter produced an abrupt rise of pressure in the opposite catheter on the other side of the capillary bed. This indicated *in vivo* a free communication in both directions between pulmonary artery and pulmonary vein without valvular or other gross obstruction and supplied indirect evidence that the obstructing catheter was in communication with only a small area of the capillary bed.

In view of these anatomical relationships, the pressure recorded distal to a point of obstruction in the pulmonary artery or vein should not be zero since an anastomotic circulation exists. It should theoretically be the pressure in the next collateral branch but on the arterial side blockage may lessen the measured pressure by reducing flow locally, just as on the venous side similar blockage may raise pressure by local passive congestion.

Gomez (11) has observed that when a systemic end artery is occluded, the pressure distal to the point of occlusion falls, not to zero, but to a point considerably above the pressure in the corresponding vein. This he refers to as the 'static pressure' of the artery. Starr and Rawson (12, 13) have likewise studied static pressure in veins. Since blood flow was maintained through all parts of the lung except in the one vessel obstructed by the catheter, static pressure could not be measured under the circumstances of these experiments.

By occluding a branch of the pulmonary artery with a catheter and a branch of a pulmonary vein in a different region with an arterial catheter, a direct connection with the capillary side of each vessel was maintained through the hole on the tip of the catheters. In this way, pressures on each side of the capillary bed, with the main vessel occluded, could be measured.

Under the conditions of these observations, pressures recorded in the obstructed pulmonary artery were lower than those recorded in the obstructed vein. This difference in pressures was not abolished by stopping respiration although there was a slight inconstant reduction in their levels when respiratory movement ceased. Immediately after blood flow had stopped (death), these two pressures became equal. Artificial respiration on these dead animals caused a slight rise of pressure in both. Therefore the differences are related largely to blood flow and only slightly to the pumping action of the lung. The discrepancy in the two pressures is probably due to the relationship of the catheters to the capillary bed, i.e. the pressure recorded through the obstructed artery is less because blood flow is reduced locally and that through the obstructed pulmonary vein is greater due to passive congestion.

It is believed, therefore, that the pressure recorded on the pulmonary artery side of the capillary bed is probably no greater than a few millimeters of mercury below



the true pulmonary capillary pressure and that on the pulmonary venous side is probably no greater than a few millimeters of mercury above the true pulmonary capillary pressure. With this reservation, pressures on the arterial side can be used to measure indirectly the direction and approximate magnitude of changes in capillary pressure. Averaging of both figures (table 1) where both can be obtained as in animals gives a means of estimating the absolute magnitude of capillary pressure.

In recording the pressure with a column of saline, the fluid runs through the catheter into the capillaries, until equilibrium is established. In recording the pressure with a Hamilton manometer, the pressure of the blood in the pulmonary capillaries is transmitted backward through the lumen of the catheter and to the membrane of the manometer. These two methods of measurement should be in close agreement. Any marked discrepancy as occasionally occurs indicates an obstruction between the catheter and the capillaries. In such circumstances, the recordings are inaccurate and should be discarded. However, it is felt that the Hamilton manometer, in general, gives the more accurate reading as equilibrium is obtained immediately while with the saline manometer equilibrium is slowly reached.

It should be pointed out that the pressures recorded in the manner described were related to a point 7.5 cm. anterior to the animals' spine when lying in the recumbent position. No attempt was made to relate the zero point to the level of the tip of the catheter which would be necessary in the estimation of the true hydrostatic pressure in the capillaries. In the majority of the dogs, however, the tip of the catheter lay about in the middle of the chest when viewed in the lateral position fluoroscopically.

It is considered that the damage found in the chambers of the left side of the heart definitely prohibits the use of arterial cardiac catheterization in man.

#### SUMMARY

1. A venous catheter was introduced into the pulmonary artery of dogs and wedged into a distal ramification so as to obstruct its lumen. Pressures beyond the point of obstruction were recorded through a hole in the tip of the catheter with a Hamilton manometer and with a saline column.

2. An arterial catheter was introduced by way of the carotid artery and left side of the heart into a pulmonary vein in similar fashion and pressures were similarly recorded.

3. In dogs, the pressures recorded with a Hamilton manometer through the catheter blocking the pulmonary artery varied between 5 and 8 with an average of 6 mm. Hg while those measured simultaneously through the catheter blocking the pulmonary vein varied between 6 and 14 with an average of 10 mm. Hg (table 1). The former is probably lower than true pulmonary capillary pressure by the 'velocity factor' and the latter too high by this same factor. It is suggested that true mean capillary pressure with unobstructed flow normally lies between these two figures, the average in 6 animals varying from 5.5 to 10 mm. Hg with an average of 8.3 mm. Hg.

4. It is considered that damage to the left side of the heart from arterial catheterization prohibits its use in man.

We wish to express our gratitude to the Godfrey M. Hyams Trust for their generous contribution for equipment used in this study.

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# HEMORRHAGIC HYPOTENSION IN HEPATECTOMIZED AND BILATERALLY NEPHRECTOMIZED HEPATECTOMIZED DOGS<sup>1</sup>

JOHN J. REINHARD, OTTO GLASSER AND IRVINE H. PAGE

*From the Research Division, Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute*

CLEVELAND, OHIO

INVESTIGATIONS of hypotensive states naturally led to the study of the contributions of the kidneys and liver in the maintenance of normal blood pressure. A participation of the renal pressor system was indicated by Sapirstein, Ogden, and Southard (1, 2), Hamilton and Collins (3) and Huidobro and Braun-Menendez (4) whose observations implied a homeostatic action of renin on the arterial pressure after bleeding. A function of the liver in shock was emphasized by Fine, Seligman, and Frank (5, 6) who concluded that inadequate hepatic blood flow is an essential factor in irreversible hemorrhagic shock. Shorr, Zweifach and Furchgott (7) described what seemed to be an integrated pattern of hepatic and renal responses to hemorrhage consisting in renal liberation of a protective VEM (vaso-excitor material) and hepatic release of an opposing principle VDM (vaso-depressor material).

As a more direct approach, these organs were individually and jointly removed before prolonged periods of hypotension were induced by controlled hemorrhage. The blood removed to produce hypotension was later reinfused to observe the ability of these animals to regain normal arterial blood pressure. Another group of animals was hepatectomized and transfused with blood from dogs which had previously endured periods of prolonged hypotension due to hemorrhage.

## METHODS

Hepatectomy was performed under light ether anesthesia by the one-stage method of Firor and Stinson (8). The animals were given intravenously 250 cc. of 10 per cent glucose in physiologic saline and 125 cc. of normal dog blood during the operation. Following recovery from ether, glucose was supplied intravenously in doses of 0.25 gm. per kilogram body weight per hour (9) and the animals were not again anesthetized. In experiments on bilaterally nephrectomized, hepatectomized animals, the nephrectomies were performed first and immediately followed by one-stage hepatectomy. Since all the experiments were of short duration, the operations were done without sterile technique.

Following a 1½-hour recovery period, experimental hemorrhagic hypotension was produced by the method of Wiggers (10) and Kohlstaedt and Page (11) and the stage of shock estimated from the criteria of Glasser and Page (12). In brief, the animals were bled rapidly from a femoral artery into a reservoir containing heparin until arterial pressure of 50 mm. Hg was established. Pressure was maintained in the reservoir by a hand bulb connected with an aneroid type manometer. The shed blood in the reservoir was in open communication through rubber tubing with the femoral

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Received for publication August 23, 1948.

<sup>1</sup> This investigation was supported in part by a grant from the United States Public Health Service, Cardiovascular Study Section.

artery of the test animal. Consequently, if the arterial pressure of the animal fell below the 50 mm. Hg level, blood passed from the reservoir into the dog. Conversely, if the arterial pressure of the animal tended to rise, more blood was shed by the dog into the reservoir. The 50 mm. Hg pressure was continued for at least 90 minutes, when the pressure was lowered to 30 mm. Hg by decreasing the pressure in the reservoir and allowing more blood to flow into it from the animal. This new pressure level was maintained for 45 minutes. Following the successive phases of hypotension, blood was restored to the animal by stepwise increases of pressure in the reservoir to the control level. Kymographic records (fig. 1) were made of arterial pressure (A.P.), shed blood volume (B.V.), respirations (RESP.) and responses to epinephrine (A.) (fig. 1). Application of this method has recently been made to a large number of animals in a critical study of hemorrhagic shock by Glasser and Page (13).

In the transfusion experiments, donor animals were anesthetized intraperitoneally with sodium pentobarbital, 30 mg./kg. body weight. The donors were similarly subjected to shock and intra-arterial reinfusion. Their blood was then drawn and citrated for transfusion into hepatectomized recipients. Blood was drawn in equal volume from the opposite legs of the recipients during the transfusion so that blood volume was unchanged. The recipient hepatectomized animals were observed for possible deleterious effects of transfusion. They were then subjected to the usual period of hemorrhagic hypotension. All animals were tested with standard doses of intravenously administered epinephrine and the rise in blood pressure was referred to as epinephrine response and expressed in mm. Hg. Page (14) has suggested that among other criteria the magnitude of this response is a rough measure of the phase of shock existing in animals with hypotension.

#### RESULTS

Animals subjected to hepatectomy invariably had widely varying degrees of blood loss into the peritoneal cavity, apparently due to diapedesis through the peritoneum. This blood loss could not be expeditiously measured pre-mortem. Consequently, among other factors, the amount of bleeding necessary to produce a standard hypotension varied widely among different experiments.

A  $1\frac{1}{2}$ -hour recovery period followed the procedures of hepatectomy and hepatectomy-nephrectomy. The hemorrhagic hypotension lasted 140 minutes. Survival for all groups averaged 7 hours after hepatectomy and 2.5 hours after hypotension.

*Group I. Hemorrhagic Hypotension in Hepatectomized Dogs.* Seven experiments were performed in which hepatectomized dogs were bled to produce hypotension. The volume of blood withdrawn to decrease the pressure to the 50 mm. Hg level varied from 20 cc. to 40 cc. per kilogram body weight. Five animals were within the narrow range of 27 cc. to 33 cc. and two, 19 cc. and 40 cc. per kilogram. Blood flowed from the reservoir into the femoral arteries of several animals in order to maintain the 50 mm. Hg level. These inflows varied and are listed in table 1A. All animals survived the procedure and regained normal blood pressure levels after the intra-arterial reinfusion of shed blood. In one case 225 cc. of additional 5 per cent gelatin solution besides the shed blood were necessary to restore the pressure to normal. The epinephrine responses in the pre- and post-hypotensive periods were equal.

Survival of these animals was limited by the nature of the experiment; however, they had normal arterial pressures for periods of from  $1\frac{1}{2}$  to  $4\frac{1}{2}$  hours. Two animals required further intra-arterial infusion of 375 cc. and 175 cc. of 5 per cent gelatin solution during these post-hypotensive periods, but again, the peritoneal loss was unknown. The results are summarized in table 1A. A typical kymographic tracing is shown in figure 1.

Group II. Hemorrhagic Hypotension in Bilaterally Nephrectomized Hepatectomized Dogs. There were 6 dogs in this group. The procedure after nephrectomy and hepatectomy exactly followed that used in Group I. The amount of bleeding

TABLE I. HEMORRHAGIC HYPOTENSION

DOG NO.	BLOOD WITHDRAWN TO PRODUCE HYPOTENSION	INTAKE AT 50 MM. HG	INTAKE AT 30 MM. HG	AMT. INTRA- ART. BLOOD REPLACEMENT	SUPL. FLUID REINFUSION 5% GELATIN	SURVIVAL POST HEPATEC- TOMY	POST HYPOTEN- SION
	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.	hrs.	
A. In Hepatectomized Dogs							
1	28	1	4	19	30 <sup>1</sup>	8.5	4.25
2	30	0	0	26	0	7.5	3.5
3	33	0	7	26	20 <sup>1</sup>	7.0	3.5
5	19	11	15	19	22 <sup>2</sup>	6.5	3.0
13	27	6	3	18	0	5	1.5
14	40	2	4	24	0	7.0	2.75
15	28	5	7	12	7 <sup>3</sup>	7.0	3.0
B. In Bilaterally Nephrectomized-Hepatectomized Dogs							
4	11	0	0	11	22 <sup>2</sup>	6.5 <sup>4</sup>	3.0 <sup>4</sup>
6	19	6	0	19	23 <sup>2</sup>	5.5 <sup>4</sup>	2.0 <sup>4</sup>
20	31	0	0	26	0	6.5 <sup>4</sup>	3.0 <sup>4</sup>
17	31	0	4	27	0	6.5 <sup>4</sup>	3.0 <sup>4</sup>
16	27	2	0	27	0	5.5 <sup>4</sup>	3.25 <sup>4</sup>
18	22	3	1	17	0	7.0	1.5

<sup>1</sup> Infused intra-arterially in post-reinfusion period.    <sup>2</sup> Infused intra-arterially during hypotensive periods.  
<sup>3</sup> Blood infused in post-reinfusion period.    <sup>4</sup> Killed.

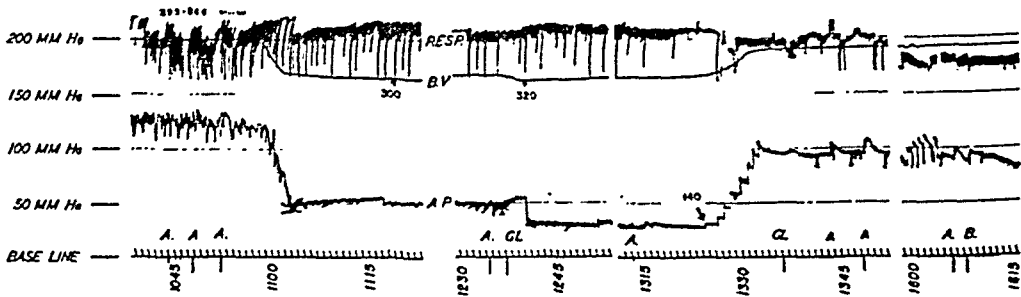


Fig. 1. Kymographic record of dog 86-6 (Sept. 16, 1947): *RESP*, respiration; *BV*, blood volume cc. in reservoir, with base line at 200 mm. Hg; *A.P.*, arterial pressure; time intervals of 1 min.; *A*, 0.1 cc. 1-10,000 epinephrine; *GL*, glucose 0.25 gm., 1 kg/hour intravenously; *B*, BaCl<sub>2</sub> 9 mg. intravenously. Hepatectomy performed at 9:30 A.M. Duration of 50 mm. hypotension, 90 min. and of 30 mm., 50 min.

necessary to produce the required hypotensive levels varied from 11 cc. to 31 cc. per kilogram body weight. Four animals were within the range of 22 cc. to 31 cc. The other two shed 11 cc. and 19 cc., respectively. Three animals took blood from the reservoir in order to maintain pressures of 50 mm. Hg. These values are listed in table 1B. All survived the periods of hypotension and were sacrificed 1½ to 3 hours after reinfusion. The blood pressure in each was restored to normal levels after

intra-arterial reinfusion of shed blood. Two received 200 cc. of additional 5 per cent gelatin solution. The responses to 0.1 cc. and 0.2 cc. of epinephrine 1-10,000 were the same in the pre- and post-shock periods. One animal required 150 cc. of 5 per

TABLE 2. HEMORRHAGIC HYPOTENSION IN HEPATECTOMIZED DOGS

DOG NO.	AMT. BLOOD TRANSFUSED FROM SHOCKED DOGS	BLOOD WITHDRAWN TO PRODUCE HYPOTENSION	INTAKE AT 50 MM. HG	INTAKE AT 30 MM. HG	INTRA-ART. BLOOD REPLACEMENT	AMOUNT SUPPL. FLUID REINFUSION	SURVIVAL POST HEPATECTOMY	POST HYPOTENSION
	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.	cc/kg.		
<i>A. Following Transfusion from Intact Shocked Dogs</i>								
7	16	3	3	3	15	13 <sup>1</sup>	5.25	1.25
8	25	13	0	3	13	0	5.25 <sup>2</sup>	1.5 <sup>2</sup>
23	23	28	7	3	27	14 <sup>1</sup>	5 <sup>2</sup>	2.0 <sup>2</sup>
19	27	40	0	7	32	0	5 <sup>2</sup>	2.0 <sup>2</sup>
25	21	19	2	8	19	3 <sup>1</sup>	5.5 <sup>2</sup>	1.5 <sup>2</sup>
24	40	13	5	3	11	0	5 <sup>2</sup>	1.0 <sup>2</sup>
<i>B. Following Transfusion from Bilaterally Nephrectomized Dogs Subjected to Hemorrhagic Hypotension</i>								
10	32	17	2	2	17	29 <sup>3</sup>	5 <sup>2</sup>	1.0 <sup>2</sup>
11	27	8	13	5	8	27 <sup>3</sup>	6	1.25
12	20	20	2	0	20	8 <sup>3</sup>	5 <sup>2</sup>	1.0 <sup>2</sup>
21	18	39	5	4	33	0	5 <sup>2</sup>	2.0 <sup>2</sup>
22	20	21	4	0	21	6 <sup>1</sup>	5 <sup>2</sup>	1.5 <sup>2</sup>
9	27	18	2	3	18	10 <sup>3</sup>	5 <sup>2</sup>	1.5 <sup>2</sup>

<sup>1</sup> Blood infused intra-arterially during hypotensive periods.

<sup>2</sup> Killed.

<sup>3</sup> Blood infused intra-arterially during post-reinfusion periods.

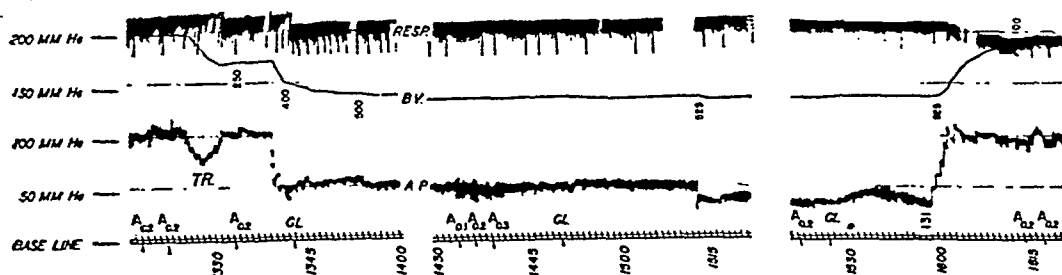


Fig. 2. Kymographic record dog 95-1 (Nov. 13, 1947): *RESP*, respiration; *B.V.*, blood volume cc. in reservoir, with base line at 200 mm. Hg; *A.P.*, arterial pressure; time intervals of 1 min.; *A*, epinephrine 1-10,000 expressed in cc.; *GL*, glucose 0.25 gm/kg. hour intravenously. Experimental procedures: hepatectomy performed at 11:00 A.M.; *TR* transfusion of 300 cc. blood from bilaterally nephrectomized shocked dog with simultaneous removal of 300 cc. of blood from opposite leg of dog 95-1 at 1325 (1:25 P.M.). Duration of 50 mm. hypotension 90 min. and 30 mm., 45 min.

cent gelatin during the post-hypotensive period in order to maintain a normal blood pressure. The results are summarized in table 1B.

*Group III. Hemorrhagic Hypotension in Hepatectomized Dogs Following Transfusion from Shocked Dogs with Intact Livers.* Six hepatectomized and 6 normal donor animals were included in the third group of experiments. Each donor animal was bled into hypotension of great enough duration to have a poor prognosis for survival

according to the criteria of Glasser and Page (5). After this was established, shed blood was replaced by intra-arterial transfusion with a pressure in the reservoir great enough to maintain normal blood pressure 10 to 20 minutes. These animals were then bled to death. The volumes so obtained (160 cc.-500 cc.) were transfused intravenously into the hepatectomized recipients while equivalent amounts of blood were withdrawn. According to our observations, these procedures had no effect upon the liverless recipients. These animals were then subjected to hypotension induced by hemorrhage in a manner similar to that of the preceding two groups. The results were not appreciably different and are recorded in table 2A.

*Group IV. Hemorrhagic Hypotension in Hepatectomized Dogs Following Transfusion from Bilaterally Nephrectomized Hypotensive Dogs.* This group of experiments was a repetition of those in *Group III* save that the 6 donor animals had been previously nephrectomized. The 6 hepatectomized recipient animals again gave no evidence of being affected by this exchange of blood. Likewise, induction of the standardized hypotensive state caused no fatalities during its course, and the arterial pressure was normal in all of the animals after intra-arterial reinfusion of the shed blood. These results are summarized in table 2B. A typical kymographic tracing is presented in figure 2.

#### DISCUSSION

A variety of altered physiological reactions—neurogenic, humoral and hemodynamic—appear during hemorrhagic hypotension. This study deals with humoral and hemodynamic factors of renal and hepatic origin since conditions of our experiments were so arranged that the neurogenic influences on the hypotensive state were believed minimal and reasonably constant.

Vasoconstriction is now generally recognized as prevailing during the onset of shock. It was attributed by Page (15) to the action of vasoconstrictor material originating in injured tissue and to vasoconstrictor impulses. Further, the caliber of the vessels seems to be indirectly influenced by their reactivity to chemical stimuli. When the responsiveness of the vascular tree is estimated by the pressor responses to epinephrine in burn shock, three phases can be distinguished: 1) the injury phase with increased arterial pressure and variably increased pressor response; 2) a transitional phase of increased pressor response followed by progressive decrease; and 3) the terminal phase in which vascular responsiveness to pressor and depressor agents is severely reduced or absent. The initial injury phase was absent in shock due to bleeding.

More recently, Shipley, Helmer and Kohlstaedt (16) have described a pressor principle which causes sustained elevation of the arterial pressure of nephrectomized, pithed cats. It is present in the plasma of sick cats, of cats poisoned with DDT and of animals subjected to hemorrhagic hypotension. Since they could not detect it in nephrectomized animals subjected to the same procedure, they consider it of renal origin. Shipley and Helmer (17) further demonstrated that the pressor principle gives only brief, unsustained increases in blood pressure in intact cats, while the responses in cats nephrectomized 48 hours before testing were invariably sustained

for several hours. These observers suggest that an inhibitor of the pressor principle is secreted by the kidneys into the blood and its concentration in blood diminishes progressively after nephrectomy. They also demonstrated that injections of pressor principle neutralizes the circulating inhibitor in recently nephrectomized animals so that repeated injections finally produce sustained responses similar to those of the 24- or 48-hour nephrectomized animals.

Hamilton and Collins (3) have shown that hemorrhage imparts pressor activity to blood as tested on nephrectomized dogs. The homeostatic capacity of renal pressor agents in shock is not large, for Bobb (18) found that nephrectomy had no effect on mean arterial pressures, survival times or rectal temperatures in dogs subjected to tourniquet shock.

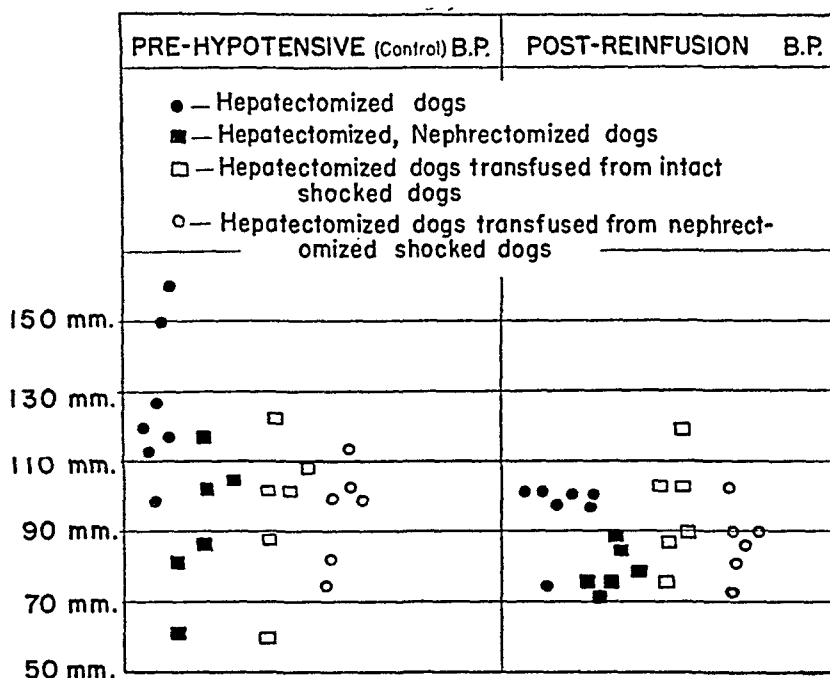


FIG. 3. GRAPH of pre-hypotensive (control) arterial blood pressures and post-reinfusion pressures of Groups I, II, III and IV.

Frank, Seligman and Fine (5, 6) concluded that loss of liver function is of crucial importance in the collapse of the organism in advanced hemorrhagic shock. These workers were able to prevent irreversible shock by vivi-perfusion of the liver of a shocked dog with the blood of a normal one. They go so far as to suggest that this is the essential effective treatment of 'irreversible' hemorrhagic shock.

Thus a summary of recent investigations indicates that *a*) experimental shock exhibits an early compensatory phase associated with vasoconstriction and frequently with increased vascular responsiveness followed by a late decompensatory phase associated with vasodilatation (Page and Abell, 13) and loss of pressor responsiveness; *b*) vasoconstrictor or vasoexcitor materials are present in the circulating blood of shocked animals; *c*) the kidney is the site of origin of a sustained pressor material on the one hand, and possibly of a distinct vasoexcitor substance (VEM) as well as a



source of anti-pressor material; and *d*) the integrity of the liver is considered absolutely necessary if the organism is to withstand shock. Our observations stand in contrast to some of these views.

We believe it desirable to avoid the terms 'shock' or 'irreversible shock', as they apply to hepatectomized animals, while they do not in any case long survive. We shall use instead the term 'hemorrhagic hypotension', as defined above under METHODS.

Regardless of whether the liver or both liver and kidneys were removed, approximately equivalent amounts of hemorrhage were necessary to lower the arterial blood pressure to the selected levels of 50 mm. Hg for 90 minutes and 30 mm. Hg for 45 minutes. Further, the bleeding volumes of hepatectomized animals, which received large volumes of blood from intact animals and from nephrectomized animals in hemorrhagic hypotension, did not differ greatly from those of hepatectomized and hepatectomized-nephrectomized animals which were not subjected to transfusion. The fact which stands out is that these animals withstand varied procedures of hemorrhage and transfusion from animals in hemorrhagic hypotension, intact or nephrectomized. Further, appropriate transfusion of shed blood restores arterial pressure and epinephrine pressor responsiveness (fig. 3).

It was apparent from 16 dogs that if only the blood removed or less were restored to the animals intra-arterially, a normotensive state could be re-established. In 9 dogs additional fluid was necessary, both during the hypotensive states and after reinfusion. The significance of this supplement cannot be established inasmuch as we have no measure of the amount of blood lost by diapedesis into the peritoneal cavity at the time of reinfusion.

#### SUMMARY

Animals deprived of livers or livers and kidneys can be subjected to prolonged hemorrhagic hypotension and still retain the ability to re-establish and maintain for some time normal arterial pressure following intra-arterial reinfusion of shed blood. This ability is not impaired by exchange transfusion of hepatectomized animals with blood from animals in hemorrhagic hypotension both intact and nephrectomized. Thus, these direct experiments do not demonstrate a critical function of the liver or kidneys in the vascular response to a standardized hemorrhagic hypotension.

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# EFFECTS OF REPEATED ORAL DOSES OF QUININE AND QUINIDINE ON THE BLOOD PRESSURE AND RENAL CIRCULATION OF DOGS WITH EXPERIMENTAL NEUROGENIC HYPERTENSION<sup>1</sup>

EDWIN P. HIATT

With the technical assistance of INEZ GREENE AND SHELTON SPARROW

*From the Physiology Department, University of North Carolina School of Medicine*

CHAPEL HILL, NORTH CAROLINA

IT HAS been shown in this laboratory that single oral doses of the cinchona alkaloids will cause an increase in renal blood flow and glomerular filtration rate in normal dogs (7). This effect lasts for several hours and occurs without significant change in arterial blood pressure. In the investigations reported in this paper we have studied the effect of repeated oral doses of quinine and quinidine given over a period of several days on the circulation of normal dogs and of dogs with experimental neurogenic hypertension.

## METHODS

A sustained hypertension was obtained in 4 dogs by excision of both carotid sinuses and division of the cervical vago-depressor-sympathetic nerve trunk on one side and the depressor nerve of the opposite side, according to the technic of Bouckaert as described by Grimson (6). These dogs, together with several normal dogs, were observed before, during and after a period of several days in which they received two to four daily doses of 10 to 15 mg/kg. of quinine or quinidine sulfate. The experiments were carried out at different intervals varying from one to 16 months after the surgical operation.

Mean arterial blood pressure was measured by puncture of the femoral artery with a 20-gauge hypodermic needle connected through a tube filled with 5 per cent sodium citrate solution to a mercury manometer. One per cent procaine without epinephrine was injected into the tissues around the artery before the puncture was made. Readings of pressure were taken a minute or two after the needle was introduced, when the pressure seemed to be fairly steady.

The renal clearance of sodium p-aminohippurate (PAH) was determined as a measure of the effective renal plasma flow. The creatinine clearance was determined as a measure of the glomerular filtration rate. PAH concentrations in plasma and urine were determined by the method of Smith *et al.* (9) while creatinine concentrations were analysed by the alkaline picrate method of Folin and Wu (5). These agents were administered subcutaneously to obtain satisfactory plasma concentrations.

Quinine and quinidine concentrations in the plasma were determined by the method of Brodie and Udenfriend (3).

It was our usual practice to give four doses the first day and three doses on subsequent days with a 10-hour interval between the last dose of one day and the first dose of the next day. After the control observations, most of the measurements were made before the first dose of the day, at the time when plasma concentrations were at the daily minimum, but occasional measurements were made at shorter intervals after a dose of alkaloid. The observations were continued for at least two days after the drugs had been discontinued.

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Received for publication August 3, 1948.

<sup>1</sup> Aided by a grant from the Cinchona Products Institute.

## RESULTS

The results of our experiments on all 4 dogs were essentially the same and are indicated in the accompanying graphical summaries of typical experiments (fig. 1).

a. *Plasma Alkaloid Concentrations.* Most of these fell within the range of 1 to 4 mg/l. These levels were effective in causing the changes described below but were not toxic as indicated by the normal behavior and appetite of the dogs. After a few doses the plasma concentration was well maintained between doses, even during the 10-hour overnight period. Two doses a day did not maintain plasma concentrations in the effective range, but three doses a day seemed to be adequate.

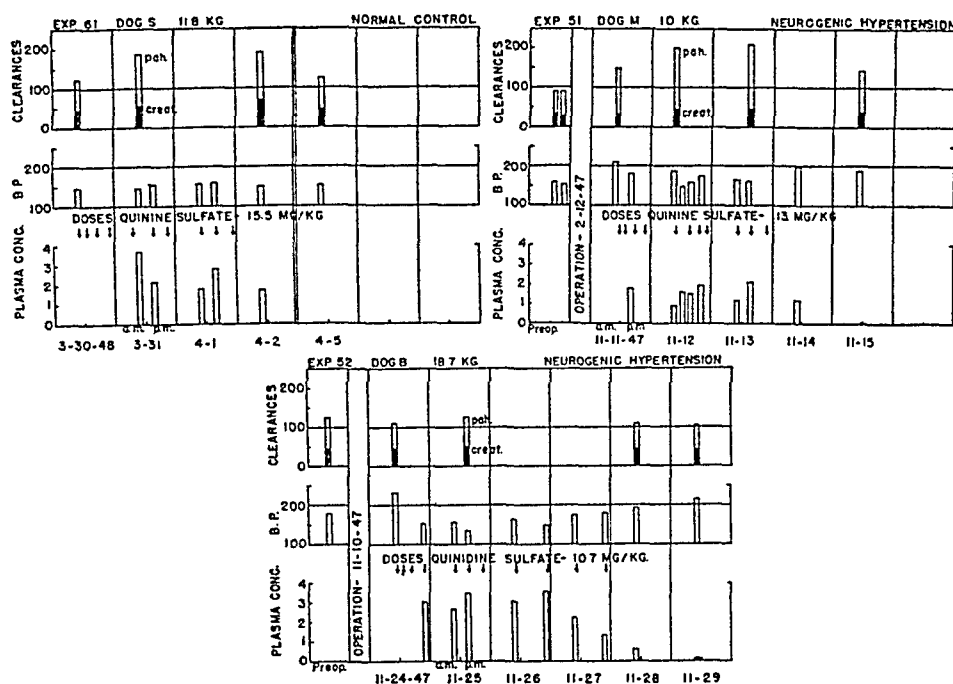


Fig. 1. GRAPHICAL SUMMARIES of three typical experiments with repeated oral doses of cinchona alkaloids. Renal clearances are expressed in cc. of plasma/min. The blood pressure is recorded as mm. of Hg mean femoral artery pressure. Plasma concentrations are shown in mg/l. of plasma. The vertical lines indicate time in days with the dates recorded on the abscissa. A. (*upper left*) Effect of repeated doses of quinine sulfate on a normal dog. B. (*upper right*) Effect of repeated oral doses of quinine sulfate on a dog with neurogenic hypertension. Preoperative data are shown on the column on the left. C. (*lower*) Effect of repeated oral doses of quinidine sulfate on another dog with neurogenic hypertension.

b. *Blood Pressure.* In the normal dogs there was little change in the mean arterial pressure. In the dogs with neurogenic hypertension there was a marked fall in blood pressure, in most cases closely approaching their normal preoperative pressures. After the second day of administration of the alkaloids the depressor effect was well maintained between doses. When the drugs were discontinued the blood pressure gradually returned to the original hypertensive levels as the plasma concentrations decreased. This recovery was usually complete in about 36 hours. Quinidine had a more marked effect on the blood pressure than quinine.

c. *Renal Circulation.* In most of the normal dogs receiving repeated doses of cinchona alkaloids, as in the experiments with single doses reported previously

(7), the renal plasma flow and the glomerular filtration rate increased, the former more than the latter. In most instances the effect lasted during the period of administration of the drug. Unlike the single dose experiments (7), the present data, with repeated doses, shows that quinine causes a greater renal hyperemia than quinidine.

Essentially the same effects on renal circulation were observed in hypertensive dogs. In spite of a considerable fall in arterial blood pressure there was no decrease in renal plasma flow and in most instances there was a definite increase, especially with quinine.

d. *Heart Rate.* In most of the normal dogs an increase in heart rate occurred after administration of the alkaloids. The hypertensive dogs which maintain a rapid heart rate (10) showed either a further acceleration of the rate or no change under the influence of the drugs.

#### DISCUSSION

In considering the mechanism by which the cinchona alkaloids reduce the blood pressure of our dogs with neurogenic hypertension there is not much help to be found in the literature. Most of the reported experiments have dealt with the depressor effect of the alkaloids injected intravenously (which is much more drastic than when the same amount of alkaloid is administered orally) and the plasma concentration of the drugs was not measured. There was, and still is, a controversy over whether or not most of the depressor effect is due to cardiac depression or to peripheral vasodilatation. Nelson (8) who reviewed the old literature felt that the action of these alkaloids is due to peripheral vasodilatation. He thought this action to be due in part to depression of the vasomotor endings and in part to an action directly on the smooth muscle of the blood vessels.

Nelson also demonstrated the antagonism of quinine and quinidine for the circulatory effects of epinephrine. We have succeeded in verifying this in experiments which will be reported later. Dreisbach and Hanzlik (4) have studied the converse relationship, i.e., the antagonism by epinephrine of the depressor effects of intravenous quinine.

Bing, Thomas and Waples studying the circulation of dogs with experimental neurogenic hypertension found evidences of increased sympathetic tone as indicated by increased cardiac output, increased renal vascular resistance and increased blood flow through the forelimb (2). These investigators also studied the effects of the adrenolytic dioxane derivatives 883 and 933 F on dogs with neurogenic hypertension and found a delayed fall in blood pressure due largely to a diminished cardiac output with a decrease in heart rate (1).

All we can say definitely at this time is that these cinchona alkaloids cause a sustained decrease in blood pressure in dogs with neurogenic hypertension. At least part of their effect is due to vasodilatation as indicated by the renal hyperemia and the well-known cutaneous flushing (8) which follows the administration of these drugs. Independent investigation of possible effects of these drugs on cardiac output are currently being undertaken.

## SUMMARY

Oral doses of quinine or quinidine of the order of 10 to 15 mg/kg. administered to dogs three times a day for several days had the following effects: *a*) in normal dogs a sustained renal hyperemia (as measured by clearance methods) without much change in blood pressure; *b*) in dogs with neurogenic hypertension, a sustained fall in the blood pressure without any decrease in renal circulation. With quinine there is actually an increase in renal blood flow. Quinidine had a greater depressor effect on the blood pressure than quinine. These effects were achieved with plasma concentration of cinchona alkaloid in the range of 1 to 4 mg/l.

We wish to thank Dr. Keith S. Grimson of Duke University School of Medicine for demonstrating to us his surgical method for causing neurogenic hypertension in dogs.

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# BLOOD PRESSURES OF RATS SUBJECTED TO AUDITORY STIMULATION<sup>1</sup>

ELEANOR H. YEAKEL<sup>2</sup>, HENRY A. SHENKIN, ALAN B. ROTHBALLER AND  
S. McDONALD McCANN

*From the Wistar Institute of Anatomy and Biology and the Harrison Department of Surgical  
Research, Schools of Medicine, University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

**R**ESULTS published by Medoff and Bongiovanni (1) indicated that a significant increase in systolic blood pressure, as measured by Griffith's method (2), occurred in gray Norway rats after prolonged exposure to the sound of a blast of compressed air. In view of the inference that this procedure might be a means of inducing a neurogenic elevation of blood pressure in response to an exteroceptive stimulus, we were led to repeat the experiment, using, however, a different method of measuring pressures in order that readings might be made as frequently as desired and without the use of anesthesia. In addition, direct measurements of systolic and diastolic pressures were obtained in a number of the animals under light ether anesthesia.

## MATERIAL AND METHODS

The rats were domesticated gray Norways of the Wistar Institute colony, ranging in age from 5 months to a year at the start of the experiment. Nine male and 8 female controls and 12 male and 13 female experimental rats were used. The control and the experimental groups were divided as equally as possible according to litter and sex. When there was a choice between rats with differing blood pressures, as shown by preliminary measurements, the animal with the higher pressure was put in the control group.

The method of air blasting has been described elsewhere (3). All experimental animals were exposed to the sound for 5 minutes a day, 5 days a week for 12 months. Blood pressures were taken during 2 months before air blasting and at random intervals thereafter, more frequently in the experimental rats and in the later stages of the experiment.

A tail plethysmograph, embodying modifications advocated by Shuler, Kupperman and Hamilton (4), was used. Following the suggestion of these authors the rats were not heated prior to testing but it was found helpful, as shown by Sobin (5), to warm the water in the apparatus. The animals were held in a cylindrical wire mesh cage of suitable diameter with a plunger at one end. The plunger was adjusted to accommodate the rat so that the full length of the tail protruded from the opposite end of the cage and could be inserted to the root in the plethysmograph. As an aid in keeping the animal quiet, a black cloth was wrapped around the head end of the holder. No anesthetic was used at any time.

Blood pressures were determined in the following way. Three consecutive readings were made, and if they agreed within 10 mm. Hg the average was taken as the blood pressure of the rat for that day. If the variation of the readings was greater, measurements were continued until agreement within a range of 10 mm. was reached. Occasionally, despite repeated trials, any three consecutive

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Received for publication May 24, 1948.

<sup>1</sup> Aided in part by a grant from the Samuel S. Fels Fund.

<sup>2</sup> Present address: Department of Anatomy, Hahnemann Medical College, Philadelphia.

readings might vary as much as 20 mm. if the animal was restless. The averages in such instances were compared with readings of the rat's blood pressure on other days when the variation fell within the prescribed limits and, if found to correspond closely, the former figures were included in the data. The number of daily blood pressures used in calculating the mean blood pressure of a rat over a given period of time is indicated on the tables in *column N*.

Direct blood pressure measurements under light ether anesthesia were obtained with a Hamilton optical manometer<sup>3</sup> on 11 of the control and 13 of the experimental rats.

TABLE 1. AVERAGE SYSTOLIC BLOOD PRESSURES OF RATS AT BEGINNING AND END OF EXPERIMENT

CONTROL RATS						EXPERIMENTAL RATS					
Rat and sex	2-month 'Control Period'		Last 2 months		Change	Rat and sex	Before air blasting		Last 2 months		Change
	N <sup>1</sup>	Average systolic pressure	N <sup>1</sup>	Average systolic pressure			N <sup>1</sup>	Average systolic pressure	N <sup>1</sup>	Average systolic pressure	
		mm.Hg		mm.Hg				mm.Hg		mm.Hg	
1 m	3	124	4	161	+37	18 f	3	108	4	169	+61
2 m	1	128	2	163	+35	19 f	1	105	5	161	+56
3 f	2	106	3	135	+29	20 f	3	117	3	168	+51
4 m	2	115	2	131	+16	21 f	2	99	4	150	+51
5 m	1	117	2	122	+5	22 m	2	108	3	149	+51
6 f	3	130	3	134	+4	23 f	2	104	4	154	+50
7 f	1	127	3	130	+3	24 m	2	98	4	146	+48
8 f	1	113	2	112	-1	25 m	2	123	3	170	+47
9 f	2	125	2	119	-6	26 m	2	116	4	161	+45
10 f	2	128	4	110	-18	27 m	2	111	3	154	+43
11 f	2	154	2	129	-25	28 m	1	107	1	148	+41
12 m			3	123		29 f	3	110	4	143	+33
13 m			2	136		30 f	2	139	5	171	+32
14 m			2	104		31 m	2	122	3	150	+28
15 m			2	122		32 m	3	105	4	130	+25
16 m			2	120		33 m	1	104	3	128	+24
17 f			3	114		34 m	2	132	3	155	+23
						35 f	2	117	3	137	+20
						36 f	2	118	5	126	+8
						37 m			4	160	
						38 m			4	151	
						39 f			4	170	
						40 f			4	183	
						41 f			5	170	
						42 f			3	141	
Mean....		124		127	+3	Mean..		113		154	+41

<sup>1</sup> Number of days on which blood pressure was determined.

#### RESULTS WITH THE PLETHYSMOGRAPH

Average blood pressures of experimental rats taken before air blasting was begun and of control rats during the same period are presented in table 1. Since no sex difference of importance was noted, males and females are listed together. During

<sup>3</sup> The measurements were made by Dr. William A. Jeffers, of the Hospital of the University of Pennsylvania, to whom we express our appreciation.



the control period the mean systolic pressures of the two groups of rats were as follows: for experimentals, 113 mm.; for the controls, 124 mm. Hg. The higher average pressure for the latter group reflects the policy of including in it, whenever possible, rats with higher readings.

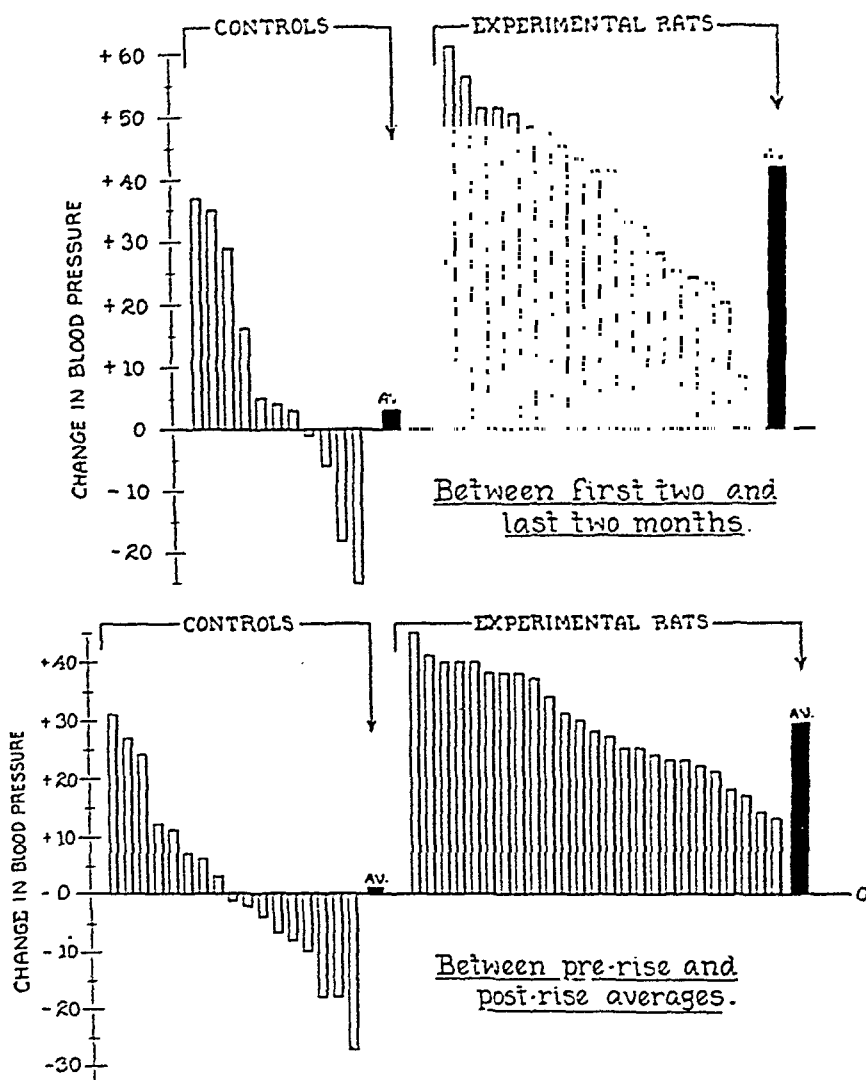


Fig. 1A (top). CHANGES IN BLOOD PRESSURE of controls and of experimental rats between first 2 and last 2 months. 1B (bottom). Changes in blood pressure of controls and of experimental rats between pre-rise and post-rise averages.

The average blood pressure of each rat in the last two months of the experiment is shown in the same table. Here the mean for the experimental animals was 154 mm. and for the controls, 127 mm. The mean rise over the control period was 41 and 3 mm., respectively, the former change being statistically highly significant ( $p < 0.01$ ). Individual alterations in blood pressures are shown in figure 1A. In all of the air-blasted rats the change was in the direction of increase and in the controls was in both directions. The frequency distribution of the average systolic pressures of the rats in the last two months shows that measurements of 150 mm. or more were found in 16 (64%) of the experimental animals and in 2 (12%) of the controls (table 3).

Inspection of the protocols showed that the increase in blood pressure in the majority of the experimental animals occurred rather abruptly, taking place in the 8th to the 11th month after air blasting was begun. When the mean blood pressure per month in the experimental group was calculated, the sharp rise was seen to occur in the ninth month of auditory stimulation, increasing thereafter to a level of 150 mm. (fig. 2). The average monthly systolic pressure in the control group was at its highest level early in the experiment and did not exceed 133 mm. in any month.

Because of the restlessness of the animals during the determinations, readings were not obtained for 6 experimental and 6 control rats during the first two months (table 1). It was thought desirable to show the average blood pressures of these rats in the early stages of air blasting for comparison with later determinations and incidentally thereby to increase *N* (the number of days on which readings were ob

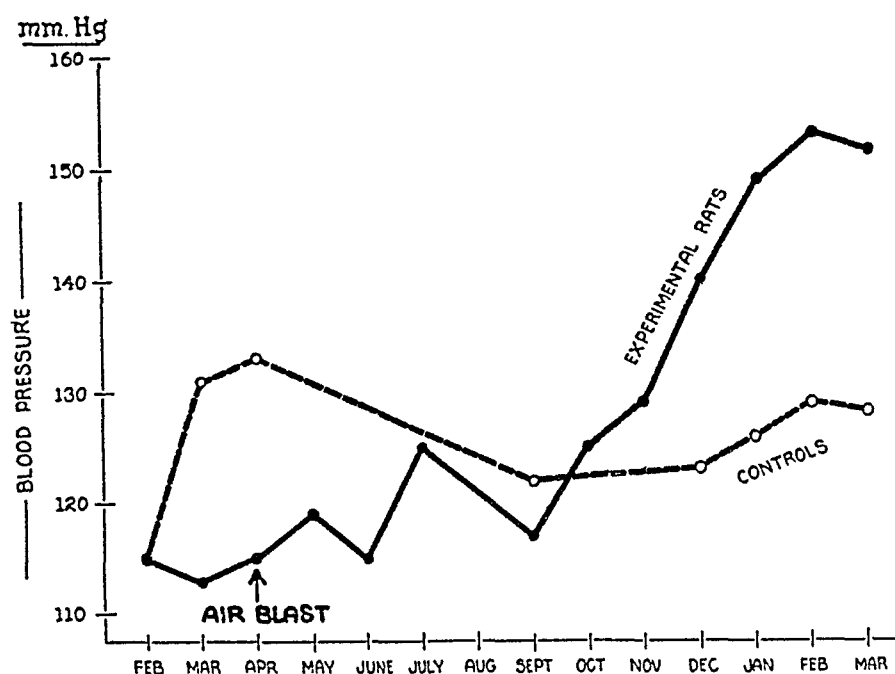


Fig. 2. AVERAGE MONTHLY BLOOD PRESSURES for one year of controls and of experimental rats

tained). Accordingly, for each control rat the readings in the 10th, 11th and 12th months were averaged, as well as the readings in the previous months. The mean systolic pressure and standard deviation of the controls for the last 3 months of the experiment was  $125 \pm 4$  mm. and for the preceding period,  $124 \pm 2.6$  mm. It may be seen that the figures differ only slightly from those given in table 1.

The readings of each air-blasted rat were scrutinized, and the measurement was noted which first indicated a rise in the animal's blood pressure. All measurements up to this one were averaged and all readings including and following it. The former are referred to in table 2 as pre-rise and the latter as post-rise averages. The individual changes are shown in figure 1B.

The mean post-rise blood pressure of the experimental group was 152 mm., an increase of 29 mm. over the pre-rise level of 123 mm. This change is statistically significant ( $p < 0.01$ ). Twelve (48%) of these rats and 2 (12%) of the controls had blood pressures of 150 mm. or greater (table 3). With this treatment of the data it

TABLE 2. AVERAGE SYSTOLIC BLOOD PRESSURES IN EXPERIMENTAL RATS BEFORE AND AFTER INITIAL RISE OF BLOOD PRESSURE

RAT AND SEX	PRE-RISE		POST-RISE		CHANGE
	N <sup>1</sup>	Average systolic pressure	N <sup>1</sup>	Average systolic pressure	
		<i>mm.Hg</i>		<i>mm.Hg</i>	<i>mm.Hg</i>
25 m	7	129	7	174	+45
20 f	9	117	9	158	+41
30 f	8	127	9	167	+40
18 f	8	130	7	170	+40
39 f	3	130	4	170	+40
26 m	7	116	12	154	+38
27 m	6	112	12	150	+38
40 f	5	141	5	179	+38
22 m	8	109	6	146	+37
21 f	11	121	3	155	+34
31 m	7	118	7	149	+31
37 m	5	139	7	169	+30
24 m	9	123	3	151	+28
34 m	7	128	3	155	+27
23 f	10	122	5	147	+25
38 m	3	123	9	148	+25
28 m	5	121	3	145	+24
42 f	8	125	2	148	+23
33 m	7	108	8	131	+23
32 m	7	118	8	140	+22
35 f	9	121	7	142	+21
19 f	7	129	7	147	+18
41 f	1	130	6	147	+17
29 f	10	115	6	129	+14
36 f	7	121	8	134	+13
Mean.....		123		152	+29

<sup>1</sup> Number of days on which blood pressure was determined.

TABLE 3. FREQUENCY DISTRIBUTION OF AVERAGE BLOOD PRESSURES

RANGE OF PRESSURES	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL
	Last 2 months of experiment		From 10th month	After initial rise in blood pressure
<i>mm. Hg</i>				
100-109	1	0	3	0
110-119	4	0	3	0
120-129	5	2	5	1
130-139	5	2	4	2
140-149	0	5	0	10
150-159	0	6	1	6
160-169	2	5	1	2
170-179	0	4	0	4
180-189	0	1	0	0

was to be expected that fewer experimental rats would be found to have readings over 150 mm. since the averages included early, relatively smaller increments of pressure. Nevertheless, the percentage of animals with elevated pressure in the experimental group is considerably higher than in the controls.

TABLE 4. BLOOD PRESSURES OBTAINED BY HAMILTON OPTICAL MANOMETER AND TAIL PLETHYSMOGRAPH

ANIMAL AND SEX	DIRECT (ETHER ANESTHESIA)		INDIRECT (UNANESTHETIZED)
	Systolic	Diastolic	Systolic blood pressure (Mean)
	mm. Hg	mm. Hg	mm. Hg
<i>Controls</i>			
1 m	175	88	161
11 f	158	67	129
12 m	155	88	124
14 m	152	95	106
8 f	146	83	103
10 f	135	85	110
5 m	130	78	123
15 m	128	80	122
9 f	115	75	119
6 f	105	60	135
3 f	98	58	135
Mean.....	136	78	124
<i>Experimentals</i>			
37 m	163	110	169
33 m	162	98	128
22 m	160	100	146
35 f	156	107	139
36 f	155	100	134
25 m	155	82	174
20 f	150	95	158
26 m	138	72	151
18 f	135	90	170
30 f	135	75	171
38 m	134	90	148
23 f	130	75	147
29 f	112	63	143
Mean.....	145	89	152

*Direct Measurements.* The data obtained with the optical manometer are shown in table 4. The average diastolic pressure of the control rats was 78 mm. Hg and of the experimental rats, 89 mm. Hg. The difference between the two groups is significant statistically ( $P < 0.05$ ). Inspection of the individual values shows that diastolic pressures of 90 mm. or more were recorded for 8 of the 13 experimental rats but for only 1 of the 11 controls. Treatment of the data by the Chi-square method gave a probability of less than 0.01 that this relation was a chance one.

The average plethysmograph readings up to the date of the Hamilton measurements are also recorded, being the post-rise averages of the experimental rats and the averages of the controls covering the last three months of the experiment. The mean of the readings in the former group is significantly higher than that of the controls. The mean systolic pressure of the experimental rats recorded directly is also higher than that of the controls but not significantly so. The agreement is poor between the indirect and direct systolic readings in the same rat. (Coefficient of correlation is 0.26.) This was thought to be the result of the different circumstances under which the blood pressures were obtained.

TABLE 5. PLETHYSMOGRAPH MEASUREMENTS OF BLOOD PRESSURE DIRECTLY BEFORE AND AFTER HAMILTON MANOMETER DETERMINATIONS IN THE SAME RAT

RAT <sup>1</sup>	PLETHYSMOGRAPH PRECEDING HAMILTON <sup>2</sup> (B)	HAMILTON (LIGHT ETHER) (A)	(A-B)	PLETHYSMOGRAPH FOLLOWING HAMILTON (LIGHT ETHER) <sup>2</sup> (B')	(A-B')
	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg
1 m c	122	127/68	+5	116	+11
14 m c	126	152/95	+26	161	-9
12 m c	101	132/75	+31	135	-3
26 m e	137	115/66	-22	107	+8
8 f c	162	146/83	-16	140	+6
10 f c	121	135/85	+14	126	+9
3 f c		98/58		106	-8
6 f c		105/60		105	
35 f e		156/107		153	+3
29 f e		112/63		114	-2
37 m e		163/110		158	+5
38 m e		134/90		205	-71

<sup>1</sup> m = male; f = female; c = control; e = experimental.

<sup>2</sup> Each figure represents the average of at least 3 consecutive readings.

This impression was verified by taking indirect measurements on a number of unanesthetized rats immediately before the Hamilton determinations and immediately after while the rats were still etherized. The data from the study are shown in table 5. It may be seen that there is poor agreement, on the whole, between the Hamilton values (*column A*) and the preceding plethysmograph results (*column B*) and that the differences are of the same order of magnitude as shown in table 4. The coefficient of correlation between *columns A* and *B* is low (0.36). There is, however, close agreement between the Hamilton and the following plethysmograph readings (*column B'*) in 11 of the 12 anesthetized rats, the difference being no greater than 11 mm.Hg in any individual animal. The coefficient of correlation here is 0.83.

#### ANATOMICAL FINDINGS

Examination of the ocular fundi in the living animals during the next to the last month of the experiment revealed no abnormalities<sup>4</sup>. At the close of the experiment

<sup>4</sup> We are indebted to Dr. Irving H. Leopold of the Hospital of the University of Pennsylvania for this information.

a number of the rats were killed, and various organs were weighed and preserved for histological examination. No changes of significance in the microscopic appearance of the adrenal glands were found. Although the average weight of the glands in the air-blasted rats exceeded the control values, the difference was not statistically significant. The weights of the cardiac ventricles were not significantly different from the expected weights calculated by Rytand's formula (6). While the average ventricular weight of experimental rats was heavier than that of control rats, statistical analysis showed that no significance may be attached to the difference.

#### DISCUSSION

The results described above verify the findings of Medoff and Bongiovanni (1) that an elevation of systolic blood pressure may be found in rats subjected to prolonged, repeated auditory stimulation. From unpublished data of their experiments we have ascertained that a nine month's exposure to the sound of the air blast intervened before the second (high) blood pressures were recorded. Apparently, under the conditions of the experiments conducted by these authors and repeated by us, approximately that length of time must elapse before a change is noted in the levels of blood pressure. It would be interesting to determine the effects of a longer daily exposure to the air blast or of an exposure oftener than once a day. In spite of the fact that the results with the control rats seem to eliminate an age change as the explanation for the higher pressures in the experimental rats, it would be desirable to bring about the result in a shorter time if only for the sake of convenience.

Direct blood pressure determinations with a Hamilton monometer failed to corroborate our previous finding of a significant increase in the systolic blood pressures of the experimental rats. However, when the blood pressure was recorded by the tail plethysmograph and the Hamilton manometer under as nearly identical conditions as was possible for us to obtain, the readings agreed quite closely. This would be expected from reports in the literature comparing the two methods (4, 5). These observations suggest that the procedures accompanying the direct method may have altered the blood pressure as found in the intact, unanesthetized rat. Traumatization and etherization, the depth of which is difficult to control, may be factors that either raised or lowered the systolic pressure. The question arises whether the diastolic pressures were not also changed by ether administration. Studies on the effects of ether upon the circulation reveal that it causes widespread peripheral relaxation, which would only serve to lower the diastolic pressure (7). Actually our data reveal a significant increase in diastolic pressure in the experimental group, as recorded upon etherized rats. This is all the more impressive in face of the over-all lowering of the systolic pressure in these same animals apparently due to the ether anesthesia and other circumstances involved in the recording of direct blood pressures.

Systolic pressures of 150 mm. or greater in 64 per cent of the air-blasted rats exceed values for normal rats reported by various authors who have used the tail plethysmograph. Readings no higher than 140 mm. in unanesthetized rats were recorded by Sulkin and Brizzee (8). The figures of Williams, Wegria and Harrison (9) show that less than 5 per cent of 1207 animals had systolic pressures of 140 to 150 mm. Loomis (10) reported 12 per cent of 100 rats with blood pressures of 140 to 150

mm. and 4 per cent between 150 and 160 mm. With other indirect methods similar results were obtained. In direct blood pressure measurements using Hamilton's optical manometer, Schroeder (11) found in 124 normal rats anesthetized with pentobarbital levels of 141 to 150 mm. in 11 animals and 151 mm. or more in 14 rats. That is, 11 per cent of his population had systolic blood pressures over 150 mm. The most stringent treatment of our data revealed an elevation of blood pressure over 150 mm. in 48 per cent of the rats.

While Grollman (12), Reed *et al.* (13), and others consider that a sustained average systolic blood pressure of 150 mm., as measured by the tail plethysmograph, indicates hypertension in the rat, we do not suggest that the elevation of blood pressure found in our experimental rats is comparable to that produced in renal hypertensive animals in which pressures of 180 mm. and higher are not uncommon and in which pathologic changes in the kidneys are demonstrable. Our experiments seem to show, however, that prolonged, repeated exposure to a stimulus that is of an exteroceptive (auditory) nature resulted in an increase in both systolic and diastolic blood pressure over the previous levels in the same rats and over the blood pressures of control rats.

#### SUMMARY

After a year's exposure to the sound of a blast of compressed air, 5 minutes a day for 5 days a week, the average systolic pressure of 25 gray Norway rats rose from an initial value of 113 mm. to 154 mm. in the last 2 months. The change is statistically highly significant. A control group of 17 animals, most of them litter mates of the experimental rats, showed an increase from 124 to 127 mm.Hg in the same period. Sixteen of the air-blasted animals (64%) and 2 of the controls (12%) had average blood pressures of 150 mm. or more at the close of the experiment.

Monthly averages indicated that the rise in systolic blood pressure of the experimental rats tended to occur in the ninth month after air blasting was begun. In the majority the increase occurred rather abruptly. The average of all systolic pressure readings in each rat before and after the rise showed an average change of + 29 mm. The difference is statistically highly significant. No such change was observed in the control group.

A significant increase in diastolic pressure (recorded under ether anesthesia with a Hamilton manometer) was found in a number of the experimental rats. There was no correlation of direct systolic blood pressure readings taken under ether anesthesia and indirect readings taken on the same but unanesthetized rats. There was a good correlation of indirect and direct systolic measurements when taken under as nearly identical circumstances as possible.

The results of the experiment suggest that an auditory stimulus applied over a prolonged period of time may produce an increase in the systolic and diastolic pressure of the rat.

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# ADRENALECTOMY AND BLOOD PRESSURE OF RATS SUBJECTED TO AUDITORY STIMULATION

S. McDONALD McCANN, ALAN B. ROTHBALLER, ELEANOR H. YEAKEL  
AND HENRY A. SHENKIN

*From the Wistar Institute of Anatomy and Biology and the Harrison Department of Surgical  
Research, Schools of Medicine, University of Pennsylvania*

PHILADELPHIA, PENNSYLVANIA

PREVIOUS investigations on the effect of prolonged auditory stimulation in rats indicated that an elevation of blood pressure resulted in the majority of the animals (1, 2). It seemed possible that the experimental procedure constituted a chronic alarming stimulus and produced the increase in blood pressure through the pituitary-adrenal cortex mechanism (3-5). As a step toward testing this hypothesis, rats with elevated blood pressures following prolonged air blasting and a group of control animals were subjected to adrenalectomy and subsequent replacement therapy. If the elevation in blood pressure was mediated by the adrenal cortex, the blood pressures of both groups would be expected to return only to normal on replacement therapy. If, on the other hand, adrenal cortical overactivity was not responsible for the elevation of blood pressure in the experimental animals, restoration therapy might be expected to produce some restoration of elevated blood pressure in the air-blasted animals. In support of this concept, some restoration of hypertension due to renal ischemia (7-15) and intracisternal kaolin (16) does occur.

## METHOD

Seven experimental and 4 control rats, ranging in age from  $1\frac{1}{2}$  to 2 years, were chosen from the groups already reported (2). The former, subjected to auditory stimulation for a year, had exhibited a sustained elevation of systolic blood pressure during the last 2 to 4 months of air blasting. The blood pressure of 3 of the controls had remained unchanged during the year, while the fourth rat had developed hypertension spontaneously. Blood pressures were taken with a tail plethysmograph without anesthesia (2). The 11 rats were adrenalectomized under ether anesthesia through the mid-dorsal approach and given 10.0 cc. of physiological saline solution subcutaneously following operation. They were maintained thereafter on one per cent sodium chloride in the drinking water. Exposure of the experimental rats to the air blasting was continued to eliminate the possibility of a fall in blood pressure due to cessation of the auditory stimulus. A prompt fall in blood pressure occurred after adrenalectomy. The blood pressures were followed for an average period of four weeks, during which time it was ascertained that a pronounced fall had occurred. Adrenal cortical extract<sup>1</sup> was then administered intramuscularly to both groups in a dosage of 1.0 cc. per animal per day for 12 consecutive days. During the time be-

Received for publication May 24, 1948.

<sup>1</sup> 'Eschatin' supplied through the courtesy of Parke-Davis Co., lot # Rx 49361.

tween adrenalectomy and the beginning of cortical extract therapy, small doses (2.5 mg.) of DCA<sup>2</sup> were given to prevent collapse from acute cortical insufficiency, the maximum for any one rat being 10.0 mg. of DCA over a period of more than two weeks. Blood pressure determinations were made every other day during cortical extract administration.

TABLE 1. AVERAGE SYSTOLIC BLOOD PRESSURES OF AIR-BLASTED AND CONTROL RATS BEFORE AND AFTER ADRENALECTOMY AND REPLACEMENT THERAPY

NO. OF RAT AND SEX	AVERAGE SYSTOLIC BLOOD PRESSURE IN MM. Hg			
	Before adrenalectomy		After adrenalectomy	
	(1) Initial	(2) After 1 year's observation	(3) Salt only	(4) Stabilized on salt & cortin
<i>Air Blasted</i>				
20 f.	117	168	117	126
22 m.	109	149	110	131
25 m.	129	170	125	119
35 f.	121	137	98	123
37 m.	139	160	92	120
39 f.	130	170	104	121
40 f.	141	183	109	137
Mean.....	127	162	108	125
<i>Control</i>				
1 m.	120	161 <sup>1</sup>	111	160 <sup>1</sup>
10 f.	117	110	77	95
12 m.	131	123	100	122
13 m.	131	136	99	118
Mean.....	125	125	97	112

<sup>1</sup> This value not included in computing mean or statistical significance.

## RESULTS

The results of the experiment are shown in table 1. The blood pressure determinations presented before adrenalectomy are averages obtained before and after the elevation in blood pressure had occurred, as explained in the previous paper (2). The average initial blood pressures of the experimental and control animals were 127 and 125 mm. Hg, respectively (column 1); during the last two months of the year's observation the averages of the air-blasted and the control rats were 162 and 123 mm., respectively (column 2), not including the one control showing a spontaneous hypertension of 161 mm. Following adrenalectomy the blood pressures of both groups fell within a week to subnormal levels; i.e., 108 and 97 mm. Hg (column 3). The blood pressures did not reach lower levels because of the small doses of DCA admin-

<sup>2</sup> 'Cortate' kindly furnished by Schering Corporation.

istered at appropriate intervals. After the administration of cortical extract the blood pressures of both groups rose in seven days or less to 125 and 112 mm. (column 4), except for the spontaneously hypertensive control, whose pressure returned to the pre-adrenalectomy value. These levels were maintained for a minimum of five days before the experiment was terminated.

The experimental data were subjected to statistical analysis. The fall in blood pressure following adrenalectomy and the subsequent rise after treatment with adrenal cortical extract were significant both in the experimental ( $p < 0.001$ ;  $p < 0.05$ ) and in the control rats ( $p < 0.02$ ;  $p < 0.01$ ). The blood pressure of the experimental animals after restoration therapy was significantly lower ( $p < 0.001$ ) than the pre-adrenalectomy figure, while there was no significant difference between comparable blood pressures in the controls. The blood pressures of both groups on cortical extract did not differ significantly from the initial values. There was no statistically significant difference between the blood pressures of the experimental and control groups when given replacement therapy after adrenalectomy.

#### DISCUSSION

From the foregoing data it may be seen that in no instance was there any restoration of elevated blood pressure in the adrenalectomized animals except in the case of the spontaneously hypertensive control, where restoration was complete. In both the controls and air-blasted rats, cortical extract did produce a rise in blood pressure to normal levels.

It has been shown repeatedly that after adrenalectomy cortical extract is capable of partially restoring hypertension due to renal ischemia (7-15) and intracisternal kaolin (16). Two reports were found in the literature (17, 18) giving relatively negative results. In none of the papers in which the dose of cortical extract was specified did the dose exceed that used here.

Since comparable doses of adrenal cortical extract produced to a degree restoration of renal hypertension after adrenalectomy and only returned the blood pressure of our animals to normal, it is suggested that adrenal cortical overactivity may play some essential rôle in audiogenic hypertension. The elevated blood pressure of the spontaneously hypertensive control apparently did not depend on this mechanism.

In experiments by Selye (5) hypertension and nephrosclerosis similar to that produced by DCA overdosage have resulted from the exposure of rats to chronic alarming stimuli; i.e., cold, formalin and exercise. Auditory stimulation is not known to be a physically damaging agent, and the exercise with occasional seizures resulting from the excitement did not seem to be of the same magnitude as that used by Selye. Also high salt intake was necessary to sensitize his animals to the effects of exercise. Thus it seems improbable that exercise was the alarming stimulus in our experiments, but fear has been considered a potent alarming stimulus (4) and may have been the initiating factor here.

The adrenal medulla has not been ruled out as a possible source of the elevated blood pressure in these animals. Because of the difference in response of these animals from those with renal ischemia, it is likely that the kidney is not primarily involved in this type of experimental hypertension, but the possibility of secondary renal involvement as suggested by Selye and Stone (6) must be considered.

## SUMMARY

To investigate the possible rôle of the adrenal cortex in the production of elevated blood pressure after prolonged auditory stimulation, adrenalectomy, with subsequent restoration therapy using adrenal cortical extract and sodium chloride, was performed on a group of experimental and control animals. Adrenalectomy lowered the blood pressure of both groups to subnormal levels. Replacement therapy with cortical extract and salt resulted in a return to normal systolic pressure in both groups. Hypertension was restored only in the case of a spontaneously hypertensive control. It is suggested that the adrenal cortex mediated the elevation of blood pressure occurring during prolonged auditory stimulation.

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# ASYSTOLIC ARTERIAL PRESSURE GRADIENT AS A MEASURE OF LOCAL PERIPHERAL RESISTANCE<sup>1</sup>

ARNOLD H. WILLIAMS AND HENRY A. SCHROEDER

*From the Department of Internal Medicine and the Oscar Johnson Institute for Medical Research, Washington University School of Medicine, and Barnes Hospital*

ST. LOUIS, MISSOURI

PERIPHERAL resistance may be defined as the frictional force opposing blood flow from the arterial to the venous system. The total force is composed of several resistances offered by various sizes of vessels, i.e., arteries, arterioles, capillaries and veins. Of these, the principal resistance results from the draining arterioles and is a function of blood viscosity and arteriolar diameter. There are a number of regional shunt pathways between the arterial and venous system, which are arranged in parallel and are related to total peripheral resistance (TPR) by the equation (1, 2):  $\frac{1}{TPR} = \frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_3}$ , etc., in which  $R_1$ ,  $R_2$ ,  $R_3$ , etc., refer to resistances of local circulations in various organs and areas of the body.

It is well known that while peripheral resistance may change equally throughout the body, territorial distribution of resistance may vary without altering TPR. It therefore seemed important to attempt to measure the relative resistances of these local circulations, in order to evaluate the part each might play in the total peripheral resistance. Of special interest were the changes which might occur in local areas under the influence of vasoactive drugs, temporary cessation of blood flow and altered physiological states such as hypertension and shock.

We have defined the asystolic arterial pressure gradient as the descending curve of intra-arterial pressure fall following sudden occlusion of a major artery supplying a local circulation. This report concerns an evaluation of the validity of the use of the gradient as a measure of local peripheral resistance; subsequent reports will deal with methods of measurement of the gradient and its use in altered circulatory states.

## METHOD

Twenty-five dogs have been used and several hundred curves were obtained. The dogs were anesthetized with nembutal (0.02 gm/kg. intravenously) followed by smaller doses given as necessary to maintain anesthesia. Arteries supplying single circulations were chosen, i.e., the brachial, femoral, superior mesenteric and renal, in order to provide major portions of the circulation for measurement. Of other major areas, the carotid was not used because of the extensive collateral circulation to the brain in the dog. A 22- or 24-gauge needle connected to a Hamilton optical manometer was inserted into the lumen of the artery chosen, directed distally, and the artery occluded at a point proximal to the needle. When the renal, femoral and brachial vessels were used, this point was usually within 3 cm. of the needle; in the case of the superior mesenteric, the occlusion was made near its origin and the

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Received for publication August 3, 1948.

<sup>1</sup>This investigation was supported by a grant-in-aid from the National Institute of Health, U. S. Public Health Service.

needle, directed proximally, tied into a small branch close to the intestine. The occlusions were made to coincide as nearly as possible with the peak of systole. The subsequent intra-arterial pressure changes, distal to the point of occlusion, were recorded on a photokymograph for about 8 to 12 seconds, and the occlusion then removed. Occlusions were sometimes accomplished by digital pressure or ligature but a rubber padded hemostat was found to be more satisfactory. To avoid mechanical artifacts, it was very important that changes in the relative positions of the artery to the needle be prevented during the gradient determination, and that the artery be as well exposed for the occlusion as experimental conditions permitted. A period of no less than 15 seconds between one occlusion and the next was found to be advisable. Systemic blood pressure was sometimes measured simultaneously from another artery with a second Hamilton manometer.

Measurements in unexposed arteries were made in a few dogs before and after anesthesia was induced. They were also made in several human subjects, using the unexposed brachial or radial artery, and occluding the arterial supply to the arm by means of a pneumatic cuff inflated rapidly from a large reservoir.

In 6 experiments gradient determinations were correlated with simultaneous measurements of femoral or brachial blood flow. The arteries were cannulated and blood flow measured by a recording rotameter (3) which was placed in a circuit between one femoral artery and the other or between the carotid and brachial arteries. Gradients were measured with the 'arterial needle' placed in the connecting rubber tubing as close to the cannula as possible with occlusion just proximal to this point. Simultaneous measurements of systemic blood pressure were made with another Hamilton manometer from the carotid or femoral artery. As an anticoagulant pontamine fast pink (type DL—Dupont) combined with heparin in normal saline was injected slowly by vein. The initial dose was 150 mg/kg. and 1 mg/kg., respectively, with subsequent hourly sustaining doses of 100 mg. pontamine plus 1 mg. heparin. In these and in other experiments, one or two wire tourniquets were placed about the limb, near the axilla or in the mid-thigh region, beneath the respective artery, vein and adjacent large nerves, in order to occlude collateral circulation.

All records were measured, at least twice, in a specially constructed grid in which the pressure was determined at accurate eighth-inch intervals. As different photokymographs and film speeds were used, this permitted measurements to be made every 0.12 to 0.7 second and linear plots of the curves to be constructed. The accuracy of the method was found to be  $\pm 2$  mm. Hg on repeated measurements.

The gradients were measured in the following manner: the mean blood pressure of the cardiac cycle immediately preceding that of the gradient was used as the initial pressure of each gradient (cf. below). The point at which a smooth descending curve began was used as the starting point from which measurements of pressure and post-occlusion time were made. Characteristics of the curve within the first 8 seconds after occlusion were usually studied. The methods of quantitating and expressing changes of resistance will be discussed in another report.

## RESULTS

Figure 1 exemplifies a typical response of the femoral arterial gradient to the intravenous injection of 100  $\gamma$  of epinephrine. Blood flow and systemic blood pressure were measured simultaneously. Mean blood pressure rose abruptly following epinephrine and during its decline femoral vasodilatation was indicated by increased blood flow and a lowering of the gradient. Later, when mean systemic blood pressure was almost the same, femoral vasoconstriction was shown by a decrease in femoral blood flow and a rise of the gradient. Figure 2 shows representative femoral gradient responses obtained before and after injection of epinephrine and later of sodium nitrite. No attempt was made to control collateral circulation in this experiment. These examples are illustrative of the responses obtained in most experiments. Thus it becomes evident that the asystolic arterial pressure gradient can be used as an index of changes in local peripheral resistance.

The greatest part of the pressure fall of the gradient (65-85%) occurred during the first 1.5 seconds. This portion was arbitrarily designated as the  $\alpha$  section. The second portion declined more gradually and was defined as the  $\beta$  section of the gradient. Regional  $\alpha$  and  $\beta$  differences appeared in the records. The renal and mesenteric arterial gradients showed an early rapid fall of pressure in contrast to a more gradual decline in the femoral and brachial arteries (fig. 3). In each instance, however, the pressure fall continued in all determinations for as long a period as we have measured (2 minutes).

#### DISCUSSION

The diastolic slope of arterial pressure has long been known to reflect changes of total peripheral resistance, being diminished when resistance is high and increased when it is low (1, 4). Following arterial occlusion the subsequent intra-arterial hemodynamics depend on several factors. The propulsive force acting to empty blood from the arterial segment is at first chiefly dependent upon the pressure prevalent at the instant of occlusion and then is sustained by the elastic recoil of the arterial wall. The force resisting draining is due principally to the frictional resistance of the arterioles imposed upon the outflowing blood. Both arteriolar diameter and blood viscosity interact to produce this resistance. Thus, with the inflow cut off, the blood volume within the arterial segment is steadily diminishing during a gradient determination. The asystolic pressure gradient delineates two pressure flow curves: the first, or  $\alpha$  gradient, under relatively physiological conditions, and the second, or  $\beta$  gradient, with a prolonged period of little flow driven by a lower pressure head at a decreasing velocity and increasing effective viscosity. At any given instant, therefore, the intra-arterial pressure and blood volume are the net results of all these forces. The influences which act to produce the shape of the curves obtained in these experiments will be considered separately.

*Intra-arterial Pressure at Instant of Occlusion.* Arterial occlusions were timed to coincide as nearly as possible with the systolic peak, but were often made on the systolic or diastolic slope. Sometimes arterial compression itself altered the initial pressure from which the gradient dropped, adding variation to that already inherent in the timing of the occlusion. On study of successive curves, however, it soon became apparent that the initial pressure was less significant than was the mean pressure at the start of the gradient. A large difference in initial pressure between two curves with the same mean pressure might shift the timing of the gradient by as much as 0.10 second but the rates of fall of pressure and the shapes of the curves were essentially the same in each case.

*Effect of Arterial Elasticity.* Once systemic blood pressure is cut off, intra-arterial pressure is directly related to the force of the elastic recoil of the arterial wall acting against the resistance of the arterioles. It is evident, therefore, that the gradient is a measure of both elasticity and resistance.

The directional changes of both arterial elastic recoil and arteriolar diameter are opposite to each other under most physiological conditions, and it is difficult to decide where the influence of one leaves off and of the other begins. Although we have not measured arterial elasticity, we believe that there is sufficient evidence to warrant

the conception that it plays a relatively minor rôle in the shape of the gradient. *a*) When the force of elastic recoil was increased by an increase in systemic blood pressure, the gradient should have shown a more rapid fall if the principal effect was due to elasticity. This did not occur. *b*) When the coefficient of elasticity was altered by drugs, the changes found were in the opposite direction to those expected if elas-

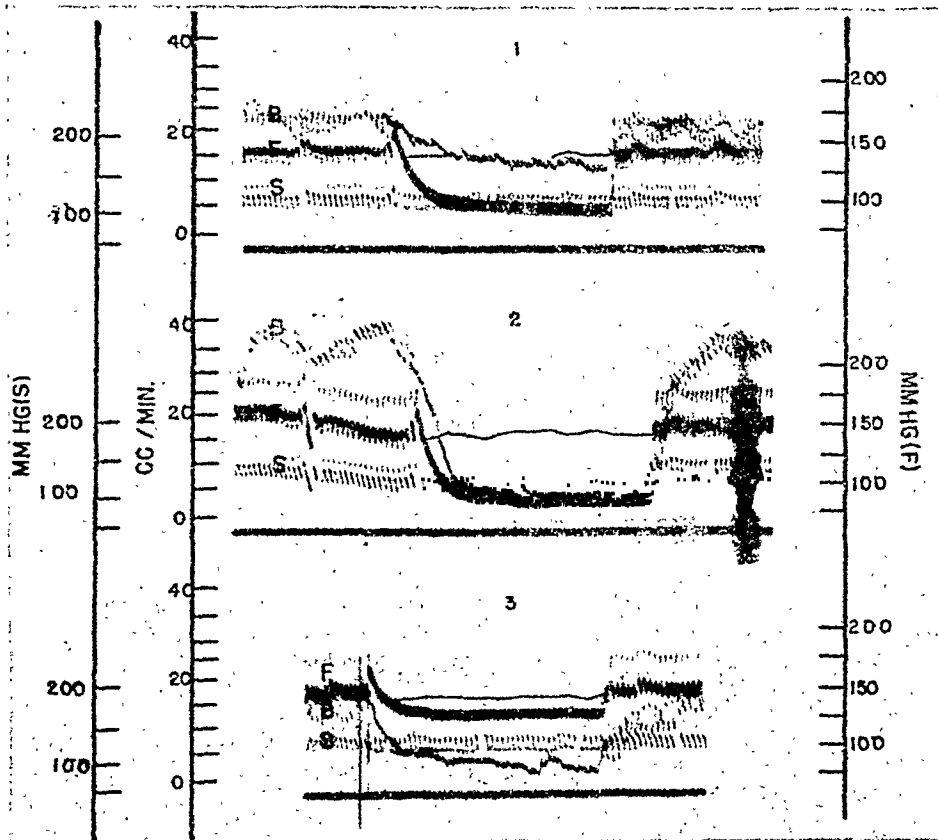


Fig. 1. SIMULTANEOUS DETERMINATIONS OF FEMORAL ARTERIAL GRADIENT AND FEMORAL BLOOD FLOW. *B* is the blood flow, measured with a recording rotameter, *F* the blood pressure of the left femoral artery, and *S* the systemic blood pressure measured in the right femoral artery. Time is indicated by interruptions of the *S* curve at 6-sec. intervals. A tight wire tourniquet was placed about the limb, occluding collateral circulation. The *S* systolic pressure has been marked manually, in order to intensify it. Curve 1 was obtained 48 sec. before the intravenous injection of 100  $\gamma$  of epinephrine, and curves 2 and 3, 25 and 143 sec. respectively, afterwards. Vasodilatation is shown in 2 by a lowering of the gradient, an increase of *B* accompanied by an increase of *S*. Vasoconstriction is shown in 3 by a rise of the gradient, and a decrease of *B*, without change in *S* from curve 2. Due to the hydrodynamics of the rotameter, flow is not shown as falling to zero during the period of occlusion. (Dog W 25—wt. 22 lbs.)

ticity were a major factor (figs. 1-3). *c*) A femoral arterial pressure-volume curve was constructed from published data (13) shown in table 1. Analysis indicates that arterial elasticity changes relatively little at the higher pressures (150-200 mm. Hg) when elastic limits are approached, but that the slope of the curve is essentially a straight line at pressures below 140 mm. Hg. At these lower pressures the rate of change of elasticity can be assumed to be almost constant. It is with these levels of pressure that the gradient is principally concerned.

*Intra-arterial Blood Volume.* It is probable that alterations of arterial elasticity



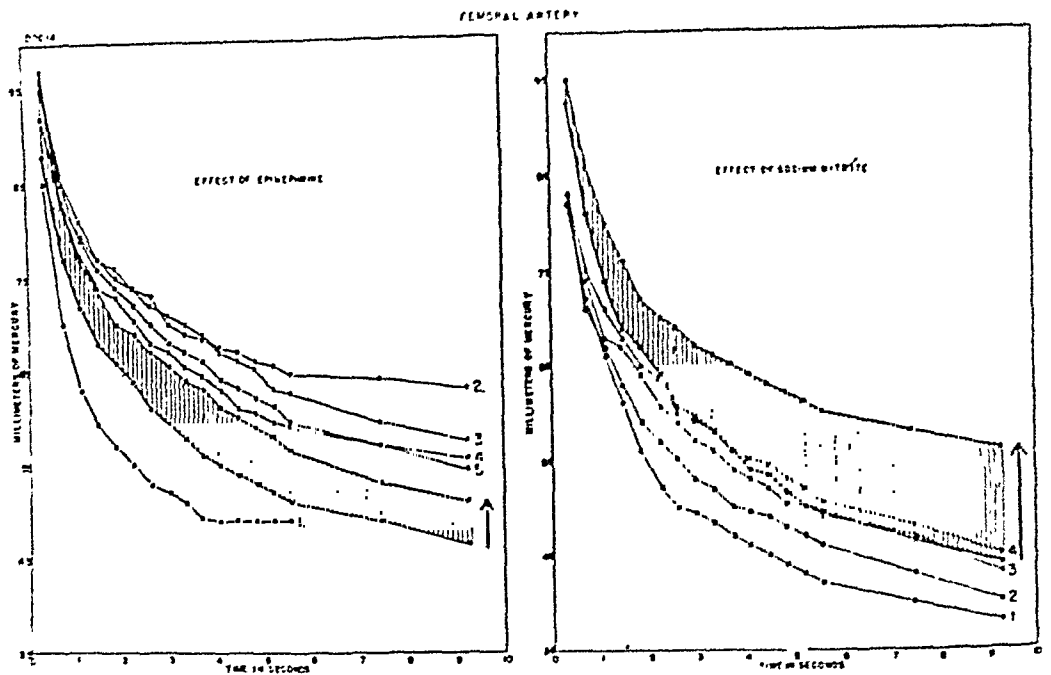


Fig. 2. REPRESENTATIVE FEMORAL GRADIENT RESPONSES. The shaded areas indicate the range of determinations of gradients (7 successive ones in A, and 4 in B) obtained at 12-sec. intervals during control periods. The arrow indicates the direction of change, the lower border being the first curve and the upper the last. Thus it is probable that vasoconstriction occurred although no attempt was made to control collateral circulation. A shows curves, numbered 1-5, obtained 30, 56, 81, 105 and 121 seconds after the intravenous injection of 100  $\gamma$  of epinephrine. It would appear that a vasodilatation occurred, synchronous with the rise of systemic blood pressure, followed shortly by a progressively diminishing vasoconstriction while the pressure remained elevated. B shows curves, numbered 1-4, obtained 59, 79, 97 and 110 seconds, respectively, following the intravenous injection of 0.1 gm. sodium nitrite. An initial vasodilatation occurred which progressively diminished while systemic pressure fell continuously. (Dog IV 14—wt. 30 lbs.)

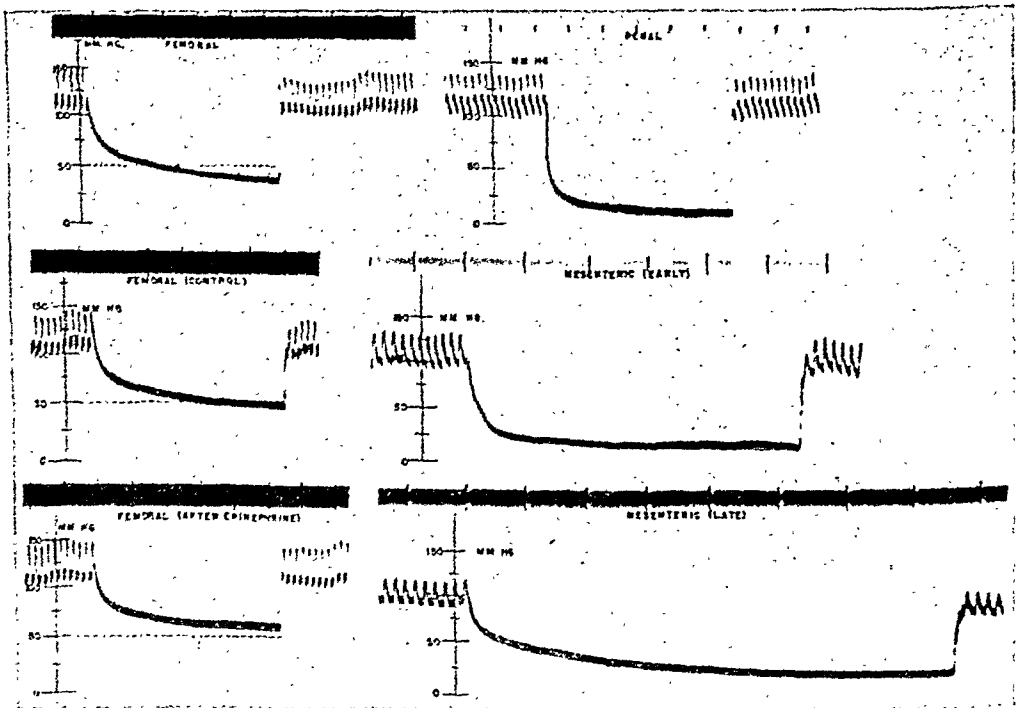


Fig. 3. RECORDS OF REGIONAL GRADIENTS. Photokymographic records of the gradients obtained from different arteries. On the left are curves obtained from the femoral artery (dog IV 14) under various conditions, the middle being the control. The lower curve was made 56 sec. after the

do not cause great variations of femoral intra-arterial blood volume (table 1). The pressure-volume curve therefore can be used as an approximate index of blood flow under the conditions of gradient determination. Assuming that the pressure is related to the fluid content of the system at a specific instant, then because there is no inflow, the change of pressure during a given time is a measure of blood outflow.

*Blood Viscosity.* Blood viscosity has been assumed to remain constant under the experimental conditions. Presumably the effect of viscosity increases as flow decreases during prolonged arterial occlusion. The action of epinephrine in increasing hemoconcentration, and therefore viscosity, may influence our results, but this influence is probably small and may be absent when other drugs are used.

*Repeated Determinations.* Repeated gradient determinations, i.e., arterial occlusions, appeared to cause a slight degree of femoral, brachial and mesenteric vasoconstriction if performed at too short intervals, that is, less than 15 seconds apart (fig. 2). These were quickly reversible. If arterial occlusions were greatly prolonged marked vasodilatation occurred, presumably from reactive hyperemia. Reversible renal vasodilatation was generally found after rapidly repeated determinations. Dicrotic notches were not seen in any of the curves, even when marked vasospasm was induced.

*Collateral Circulation.* The contour and rate of pressure fall of the asystolic arterial pressure gradient may be markedly influenced by collateral circulation, except possibly in the kidney. Under normal conditions collaterals do not supply blood to these cognate<sup>2</sup> vascular beds as there is no pressure differential between the collateral and cognate systems. Intra-arterial pressure ( $P_A$ ) falls in the cognate system during the gradient determination while the pressure within the collaterals ( $P_C$ ) remains unchanged. Therefore, the differential pressure,  $P_C - P_A$ , increases proportionately to the fall of  $P_A$ . This differential pressure head and content of blood within the collaterals is transmitted into the cognate system in amounts proportional to the diameter of the collaterals and the magnitude of  $P_C - P_A$ . The amount of this blood flow may be considerable (5) and may increase with repeated gradient determinations.

The presence of collateral circulation is detected by *a*) pulsations in the curve synchronous with the pulse rate, *b*) a rise of pressure during the gradient determination and *c*) an increase of gradient pressure levels in sequential curves of a control period. Despite these facts it seems probable that the asystolic gradient is a measure of local peripheral resistance when the curve shows a smooth progressive non-pulsatile fall. There are several lines of evidence for this view. *a*) Gradient changes were found following the injection of a vasoactive drug in the absence of any change in systemic blood pressure. *b*) Gradient changes have been shown to occur in a direc-

<sup>2</sup> Cognate denotes the direct lineal arterial pathways as contrasted to collateral pathways (5).

intravenous injection of 100  $\gamma$  of epinephrine, and the upper made 7 min. later. Note that the change in systemic blood pressure, indicated on the scale at the left, was relatively less than the change in the shape of the gradient. The curves obtained from the renal (upper right—dog W 13) and mesenteric arteries (middle right—dog W 19) show a more rapid fall during the  $\alpha$  gradient period than do those from the femoral. The lower right curve (dog W 19) was made about 2 hours after the middle one, when the animal was in a state of incipient shock. Note that the shape has changed considerably and that the systemic blood pressure is lower. Time is indicated at 2-sec. intervals along the abscissa. No attempt was made to control collateral circulation in these experiments.

tion opposite to the change of systemic blood pressure (figs. 1 and 2A), which is contrary to what might be expected from the  $P_C - P_A$  differential. c) Similar curves have been obtained following the action of a drug whether or not collateral circulation has been prevented (compare figs. 1 and 2A). d) In some instances, when collaterals were obvious, typical gradient changes occurred following the action of vasoactive drugs. e) Presumably the diameter of the collateral channels are influenced similarly to the arterioles by vasoactive drugs.

In two experiments (nos. W-29 and W-30), with conspicuous collateral blood flow into the cognate bed, progressive ligation of all visible collaterals of the widely exposed femoral artery was accomplished, the process being interspersed with gradient determinations. Later wire tourniquets were applied and a comparison made of the

TABLE 1. CALCULATION OF THE VOLUME OF BLOOD IN THE FEMORAL ARTERY OF A 15-KG. DOG (0.5 SQ. M. BODY SURFACE)

PRESSURE	CC. BLOOD VOL. IN BOTH LEGS / SQ. M. BODY SURFACE	CALCULATED CC. BLOOD VOL. IN FEMORAL ARTERY
0	46.3	8.68
20	48.0	9.00
40	50.3	9.43
60	52.5	9.84
80	55.0	10.31
100	56.8	10.65
120	58.6	10.99
140	60.3	11.30
160	61.5	11.53
180	62.3	11.68
200	62.9	11.82

Data calculated from table 2 of the paper by Remington and Hamilton (13). The above values are probably high for femoral blood volume as pelvic blood supply was included in the original calculations. The femoral volume was considered to be three fourths of the total of that of a single hind leg.

gradients taken under the two conditions. The gradient fell more abruptly and the  $\beta$  section was at lower pressure levels after the tourniquets had been tightened.

There are no absolute criteria of vasoactivity in the determination of the gradient when collateral circulation is present, unless simultaneous measurements of blood flow are made. The only conditions under which one can be certain that collaterals have no effect upon the gradient are when flow is diminished, pressure unchanged or lower, and the gradient rises, a combination of changes indicating local vasoconstriction. Similarly, when flow increases, pressure is unchanged or rises and the gradient falls, vasodilatation without collateral interference can be assured. Thus, for example, a rise of the gradient following a drug injection, when collateral circulation is not excluded, may be due to pressure and flow transmission from the collateral into the cognate system and/or vasoconstriction. However, for the above reasons we believe that the asystolic arterial pressure gradient is a qualitative measure of local peripheral resistance in the presence of collateral circulation, and a quantitative measure if collateral circulation is excluded, provided that mean pressure is taken into consideration (6).

*Effects of Prolonged Arterial Occlusion.* In some femoral and brachial gradient determinations, arterial occlusion was prolonged up to two minutes. When the initial mean blood pressure was about 100 mm. Hg, intra-arterial pressure reached venous pressure levels in about two minutes when collateral circulation was excluded. With higher initial pressures, venous pressure levels were not reached during this time. Even with collateral circulation present, gradient pressures continued to fall at the end of two minutes. Such prolonged occlusions are followed by marked vasodilatation and increase of blood flow. It is doubtful, therefore, that any change of venous pressure with such drugs as epinephrine or sodium nitrite plays a part in the form of the gradient, for such changes are extremely small (7, 8).

The vasodilatation noted after prolonged arterial occlusion, in response to anoxia or the accumulation of metabolites, may occur at some point along the course of the gradient. We have seen no evidence, however, of sudden changes in the gradual decline of the curve, which would indicate a more rapid outflow of the blood captured in the local arterial network. In two experiments in man, using photo-electric plethysmographs on the fingers, prolonged brachial occlusions were followed by relatively rapid but small decreases in finger volume occurring 25 seconds later.

Finally, a word may be said about the levels of pressure at which flow ceases. Some investigators (9, 13), using data derived from perfusion of limbs (10), have considered that effective flow is proportional to pressure minus 20 mm. Hg, which suggests that below this level flow ceases. Our experiments do not support this hypothesis. Often gradients of the renal and mesenteric systems fell to 10 mm. Hg or lower in the first 3 seconds. In a few cases femoral and brachial arterial gradients affected by vasodilating drugs fell to a range of 12 to 17 mm. Hg within 10 seconds. During prolonged arterial occlusion pressure continued to fall as long as the artery was clamped. The rôle of dilatation induced by ischemia on the continued fall of pressure is not known, but if blood flow continues as long as pressure is falling, we believe that 20 mm. Hg is not the level of pressure at which flow ceases. Therefore, effective flow should probably be considered as proportional to arterial pressure minus venous pressure. The data of others (11, 12) bears this out.

#### SUMMARY

The asystolic arterial pressure gradient has been defined as the descending curve of intra-arterial pressure following sudden occlusion of a major artery. Evidence was presented that the gradient can be used as a measure of changes in the peripheral resistance of local areas of the circulation. This measure is strictly quantitative only if collateral circulation is excluded but it may be used as a qualitative index of resistance when some collateral circulation is present.

The hemodynamic factors involved in the formation of the contour of the gradient were discussed.

We wish to express our appreciation for the technical assistance of Marie Klose and Betty D. Wheeler.

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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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Published by  
THE AMERICAN PHYSIOLOGICAL SOCIETY

VOLUME 155

November 1948

NUMBER 2

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## INVESTIGATIONS OF FREE AND BOUND POTASSIUM IN RAT BRAIN AND MUSCLE<sup>1</sup>

DAVID STONE AND SUMNER SHAPIRO

*From the Worcester Foundation for Experimental Biology*

SHREWSBURY, MASSACHUSETTS

IT IS the purpose of the present paper to investigate what fraction of the total cellular potassium occurring in rat brain and muscle is diffusible. The existence of a non-diffusible form of potassium has been suggested by several investigators. Yannet (8) discusses a potassium fraction within the cell which is not osmotically active and refers to this as a 'bound fraction'. Fenn (2), in a review of the rôle of potassium in physiological processes, cites Ernst's conclusion that stimulation of a muscle results in the breakdown of 'potassium compounds' with a resulting loss of diffusible potassium from the cell, when cell permeability is increased, and later he refers to Neuschlosz's finding that a certain fraction of muscle potassium cannot be extracted from minced muscle by isotonic sodium chloride. Pichler (5) describes a water soluble, a lipoid soluble and a third potassium fraction found in the central nervous system of frogs and Reginster (6) reports the existence of two potassium fractions, a diffusible and a non-diffusible fraction, in muscle, the former increasing with activity. Several authors discuss the nature of bound potassium. Christiansen and Hastings (1) find that with increasing  $pH$ , cephalins bind appreciable amounts of potassium and Folch (3) claims that three brain lipids—cerebron sulfuric acid, phosphatidyl serine and diphosphoinositide taken together can bind 27 per cent of the potassium occurring in brain.

### PROCEDURE

*Ultrafilters.* In the experiments that follow, an ultrafiltration apparatus was devised wherein collodion was used as a semipermeable membrane. To construct ultrafilters, visking cellulose sausage casing (20/23" inflated diameter) was cut into six-inch strips and washed until sodium and potassium free. Strips were knotted at one end and tested for leakage, then passed over No. 2 rubber stoppers which had been fitted on separatory funnels, and bound tightly in place with washed No.

Received for publication September 10, 1948.

<sup>1</sup> This work was aided by Contract N6 ori-197 of the Office of Naval Research.

12 Cuddyhunk. The diameter of the Cuddyhunk is important; if too small, tight binding will tear the collodion; if too large, the binding will not be secure.

Linen or down-proofing bags which had been boiled and washed were secured around the collodion bags to prevent their exploding under pressure and the filters thus constructed were fitted into side-arm suction flasks and tested for leakage with distilled water under one atmosphere air pressure. To prevent leakage it is essential that the collodion be kept moist after once being wet. Liquid collodion was used to make air-tight seals between glass and rubber connections.

*Ultrafiltration of control solutions.* Control solutions containing known concentrations of sodium and potassium chloride were ultrafiltered for 12 to 18 hours under one atmosphere air pressure at 3°C. The ratio of the concentration of sodium and potassium cations passing through the filter to the concentrations of cations in the control solutions was determined by the Perkin-Elmer flame photometer (model No. 18).

*Ultrafiltration of tissue homogenates.* Male white rats weighing ca. 200 gm. were killed by ether and the brain (sans cerebellum) and left gastrocnemius muscle were quickly removed, weighed wet and each then mashed for four minutes in the Waring blender in 100 or 200 cc. of cold distilled water. Mashing was carried out at 3°C.

Homogenates of brain and of muscle prepared in this manner were divided into three portions. Two of these were ultrafiltered under the same conditions as were the control solutions (see above); the third portion was stored 12 to 18 hours at 3°C. Potassium determinations were made on all three solutions by the flame photometer for a comparison between the filtered and non-filtered aliquots. Usually a further dilution to 1/300 was necessary for bringing readings into the range of our calibration curve of the flame photometer.

Whereas brain homogenates could be analyzed directly, the estimations of potassium concentrations in the non-filtered muscle solution were thought to be inaccurate, for muscle homogenates prepared on the Waring blender characteristically contained visible strands of connective tissue. It was suspected that the photometer might not burn these strands completely and thus give low readings. As a check, one series of muscles was ashed in a muffle furnace at 550°C. for one hour, then taken up in 0.1N (potassium and sodium-free) hydrochloric acid and made up to 100 cc. volume with distilled water, while a second series of muscles was homogenized as above then boiled under a reflux condenser to prevent concentration of solutions, until the strands were broken up.

Comparisons of potassium concentrations were subsequently made between the muscle homogenates prepared by these three methods.

*Ultrafiltration boiled tissue solutions.* A less cumbersome method was devised wherein homogenization was not necessary. For determinations of the total amount of potassium contained in a tissue, we have found that an intact brain or muscle may be boiled under a reflux condenser in 100 or 200 cc. of distilled water for 10 minutes, then stored for 36 hours at 3°C. (7). At the end of this time ultrafiltrations of the liquid surrounding the fragmented tissue were carried out and potassium concentrations were determined simultaneously in both filtered and non-filtered aliquots of this fluid.

*Refiltration of filtrates.* As controls of the method the filtrates of several homogenates were aliquoted and passed through a second ultrafilter. Conditions of the second filtration were identical with those of the first.

*Sodium determinations.* Wherever possible, the sodium concentrations of filtered and non-filtered aliquots were made concurrently. Sodium determinations were made at dilutions of 1/100.

## RESULTS

*Ultrafiltration of control solutions.* In all cases where control solutions containing known concentrations of sodium and potassium were ultrafiltered, the ratio: Concentration of cation in ultrafiltrate/Concentration of cation in reagent solution was close to unity. Discrepancies which were reported fell within the 5 per cent experimental error which is characteristic of our flame photometer.

*Ultrafiltration of tissue homogenates.* A mean value of  $3.47 \pm 0.09$  mg. K/gm. wet brain was found in the nonfiltered aliquots of brain homogenates. In the case of muscle, three different mean values were derived from non-filtered aliquots prepared by the three methods described above. The highest of these was  $3.87 \pm .06$

TABLE 1. MEAN VALUES OF BRAIN AND MUSCLE POTASSIUM CONTENT MG/GM.

	BRAIN	MUSCLE		
	Homogenized	Homogenized	Homogenized boiled	Ashed
	A	B	C	D
Mean values.....	$3.47 \pm .09$	$3.56 \pm .07$	$3.87 \pm .06$	$3.74 \pm .04$
No. of expts.....	12	12	11	8

Comparing columns B and C,  $t = 3.3$ ,  $P = 0.01$ ; differences significant.

mg. K/gm. wet muscle derived by boiling muscle homogenates. These values are presented in table 1.

Application of Fisher's *t*-test shows that there is a statistically significant difference between the mean 'homogenized' and mean 'homogenized-boiled' muscle values,  $P$  being 0.01.

For subsequent calculations on the percentage of diffusible potassium occurring in muscle, this highest value,  $3.87 \pm .06$  mg. K/gm. wet muscle, was used as a standard denominator.

Analysis of the ultrafiltered aliquots of brain and muscle showed that 75 per cent and 72 per cent respectively of the total potassium were diffusible through collodion. In the case of brain this fraction was determined by comparing the concentration of potassium in the homogenized filtered aliquot to the concentration of potassium in the homogenized-boiled, non-filtered aliquot. Table 2 summarizes these results.

A mean value of  $3.42 \pm .08$  mg. K/gm. wet brain and  $3.96 \pm .12$  mg. K/gm. wet muscle were found by analysis of non-filtered boiled tissue solutions. These values do not vary significantly from total potassium determinations made on tissue homogenates as shown above. Comparisons are made in table 3.



Ultrafiltered aliquots of boiled tissue solutions showed 69 per cent and 70 per cent of diffusible potassium in brain and muscle respectively.

Table 4 presents a comparison between the percentage of non-diffusible potassium in brain and muscle as determined by the two ultrafiltration methods described. Application of Fisher's *t*-test shows that there is no statistically significant difference

TABLE 2. MEAN VALUES, TOTAL AND NON-DIFFUSIBLE POTASSIUM IN BRAIN AND MUSCLE

	TOTAL MG. K/GM. HOMOG.	MG. K/GM. HOMOG. & ULTRAFILT.	% NON- DIFFUSIBLE	TOTAL MG. K/GM. HOMOG. BOILED	MG. K/GM.	
					Homog. & ultrafilt.	Non-diffusible
	A	B	C	D	E	F
Mean values.....	3.47 ± .07	2.60	25 ± 1.74	3.87 ± .06	2.90	28 ± 2.84
No. of expts.....	12	14		11	15	

TABLE 3. MEAN VALUES OF BRAIN AND MUSCLE POTASSIUM CONCENTRATIONS AS DETERMINED BY HOMOGENIZING VS. REFLUX-BOILING WHOLE TISSUES

	BRAIN		MUSCLE	
	Homogenized mg. K/gm.	Reflux-boiled mg. K/gm.	Homogenized-boiled mg. K/gm.	Reflux-boiled mg. K/gm.
	A	B	C	D
Means.....	3.47 ± .09	3.42 ± .08	3.87 ± .07	3.96 ± .12
No. of expts.....	12	23	11	17

Comparing columns A and B,  $t = 0.42$ ,  $P = 0.5$ ; not significant.

Comparing columns C and D,  $t = 0.763$ ,  $P = 0.4$ ; not significant.

TABLE 4. MEAN VALUES, COMPARISON OF ULTRAFILTERED TISSUE HOMOGENATES VS. REFLUX-BOILED SOLUTIONS

	BRAIN		MUSCLE	
	Homogenized % non-diffusible K	Reflux-boiled % non-diffusible K	Homogenized % non-diffusible K	Reflux-boiled % non-diffusible K
	A	B	C	D
Means.....	25 ± 1.74	31 ± 2.84	28 ± 1.56	33 ± 2.05
No. of expts.....	14	18	15	19

Comparing columns A and B,  $t = 1.72$ ,  $P = 0.1$ , no significant difference.

Comparing columns C and D,  $t = 1.94$ ,  $P = 0.05$ , no significant difference.

between the percentages of non-diffusible potassium as determined by these two methods in brain or in muscle.

*Refiltration of filtrates.* The refiltration of several filtrates, in a total of six experiments, showed that a mean value of 92 per cent of the potassium by concentration in both brain and muscle would pass through a second ultrafilter.

*Sodium determination.* In a total of 14 experiments both sodium and potassium concentrations of filtered and non-filtered aliquots were obtained. Within experimental error 100 per cent of both brain and muscle sodium were diffusible while

in this same series 75 per cent of brain potassium and 78 per cent of muscle potassium were diffusible.

#### DISCUSSION

The total potassium values of brain and muscle reported in the present paper,  $3.47 \pm .09$  mg. K/gm. brain and  $3.87 \pm .06$  mg. K/gm. muscle, are in agreement with values reported elsewhere in the literature (4), as well as with values obtained by a second method herein reported.

Two methods for determining the percentage of non-diffusible potassium have been demonstrated and values of 30 per cent obtained by ultrafiltration of tissue homogenates and by the ultrafiltration of solutions in which intact brain and muscle had been boiled, show no statistically significant variation from each other; further corroboration of this figure of 30 per cent is offered by Folch (3) who reports a 27.2 per cent bound K fraction in brain.

As controls on the ultrafiltration method, we have shown that control solutions of sodium and potassium chloride can be vacuum ultrafiltered with accurate cation concentration determinations; further, the refiltration of filtrates reported, indicates that the potassium fraction which we have considered as free, is really diffusible through collodion. Repeated filtration of the free fraction consistently yields the same results; 92 per cent of the free fraction can be refiltered. The 8 per cent of the diffusible potassium fraction which is lost in the refiltration process may be due to any of several factors, but elaboration of this phenomenon would require more detailed investigation. While the collodion membrane allows passage to only 70 per cent of the total potassium, 100 per cent of the sodium filters through, thus further substantiating the reality of the bound fraction of potassium.

The nature of the potassium binding is not understood. Presumably potassium ions are associated with large thermolabile molecules. Folch (3) has attributed this binding rôle in the brain to lipids.

#### SUMMARY

To determine the percentage of diffusible potassium occurring in rat brain and muscle, an ultrafiltration process was developed where visking cellulose sausage casing served as a semi-permeable membrane. Following satisfactory ultrafiltration of control solutions, homogenates of rat brain and muscle were separately ultrafiltered. The percentage of diffusible potassium, in the case of brain, was calculated by comparing the potassium concentration in the ultrafiltrate to that of the original homogenate. In the case of muscle, the potassium concentration of the ultrafiltrate was compared to that of the original homogenate boiled. Mean values of  $3.47 \pm .09$  mg. K/gm. brain of which  $25 \pm 1.74$  per cent is non-diffusible and  $3.87 \pm .06$  mg. K/gm. muscle of which  $28 \pm 2.84$  per cent is non-diffusible were obtained. Total potassium content values of  $3.42 \pm .08$  mg. K/gm. brain of which  $31 \pm 2.84$  per cent was bound and  $3.96 \pm .12$  mg. K/gm. muscle of which  $33 \pm 2.05$  per cent was bound were obtained by the analysis of supernatant liquids in which whole tissues had been boiled.

As controls of the method, first, the refiltration of several filtrates showed that

a mean value of 92 per cent by concentration of both brain and muscle potassium would pass through a second filter and, second, within experimental error, 100 per cent of both brain and muscle sodium were diffusible while in the same series 75 per cent of brain and 78 per cent of muscle potassium were diffusible.

We thank Dr. Hudson Hoagland for his advisory assistance and Mrs. Pauline Perry for technical aid.

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# CYCLIC CHANGES IN THE RESPIRATORY CENTERS REVEALED BY THE EFFECTS OF AFFERENT IMPULSES<sup>1</sup>

MARTIN G. LARRABEE AND ROBERT HODES<sup>2</sup>

*From the Eldridge Reeves Johnson Foundation, University of Pennsylvania School of Medicine*

PHILADELPHIA, PENNSYLVANIA

IT IS the purpose of this paper to present evidence of changes in the respiratory centers occurring progressively during inspiration and expiration. The rhythmicity of natural respiration may be explained by assuming that inspiration stops when these progressive changes reach a certain critical level and starts again when another critical level is attained. An analysis of such alterations in the centers can therefore contribute to an understanding of normal respiratory mechanisms.

In experiments previously reported, it was found that in natural breathing the frequency of discharge over phrenic motoneurons was unaffected by impulses from pulmonary stretch receptors until the very end of inspiration; then impulses from these receptors caused a very rapid decline in frequency and finally a cessation of the motoneurone discharge (5). Since afferent impulses from pulmonary stretch receptors are known to be discharged at progressively increasing frequency throughout inspiration (1), these findings suggest that there may be a sharp threshold for stopping inspiration, which the afferent impulses must exceed before a detectable effect is produced. Such a sharply-defined threshold for stopping inspiration was indeed revealed by Boyd and Maaske (2) by electrical stimulation of the vagus nerves.

This well-defined threshold for stopping inspiration should be a useful tool for exploring changes in the centers during the respiratory cycle, alteration of the chemical environment of the center and under other experimental conditions. Changes during the respiratory cycle were investigated in the following experiments. During the course of the investigation it was found that further information concerning the changes occurring in the centers could be obtained by combining the study of the threshold for stopping inspiration with an analysis of the delay in start of inspiration caused by brief volleys of afferent impulses during expiration.

The results obtained by these two procedures were in general found interpretable in terms of current concepts of the organization of the respiratory centers.

## METHODS

Experiments were performed on cats anesthetized with Dial.

In order to record impulses discharged by motoneurons supplying the diaphragm, the upper root of the phrenic nerve was cut as far distally as possible without opening the thorax. The central end of the nerve was freed from the surrounding tissues and placed on recording electrodes connected

Received for publication September 2, 1948.

<sup>1</sup> Aided by a grant from the Supreme Council, Scottish Rite Masons.

<sup>2</sup> Fellow in the Medical Sciences, National Research Council, 1941-42.

to a vacuum tube amplifier and cathode ray oscillograph. For most experiments the end of the nerve was split into several fine strands and a strand was selected for recording in which only one fiber became active during each inspiration.

Respiratory movements were recorded by means of a tambour pressed against the abdomen. Electric stimuli were applied to the superior laryngeal nerve. In order to permit precise timing of the stimuli, with respect to the phrenic nerve impulses, the stimulator was keyed by the impulses themselves through a connection from the output of the amplifier. When a switch in this circuit was closed during expiration, the first impulse in the next inspiratory discharge turned on an electronic delay circuit. At the end of the delay, which was adjustable, the stimulator was automatically started and delivered a controlled number of stimuli at any desired frequency and intensity. The stimuli passed to the nerve through an isolating transformer which served to minimize spread of current. After each application of stimuli, a number of inspirations were allowed to occur normally before further stimulation, in order to avoid complications due to persisting effects of the tests in the respiratory centers.

The superior laryngeal nerves were chosen for stimulation in these experiments because it was known that afferent impulses in these nerves have a strong inhibitory action on inspiration. Moreover, it was hoped that the superior laryngeal nerves might contain fewer kinds of afferent fibers than the vagus nerves, which are known to carry afferent impulses from at least four sets of receptors subserving different respiratory reflexes.

The functions of even the superior laryngeal nerves are not simple, however, for impulses in their afferent fibers can increase as well as decrease the activity of phrenic motoneurons. This is apparent, for example, in the records reproduced in figure 1. Relatively weak stimuli (*record B*) had an inhibitory action on inspiration, indicated by a reduction in the frequency and duration of the motoneurone discharge and by a delay of the subsequent inspiration. When the strength of stimulation was increased, however, instead of a further decrease in number of impulses, there was an increase (*record C*); impulses were actually discharged for a longer time and at a higher maximum frequency than in a normal inspiration. These stronger stimuli thus initiated afferent impulses causing an excitatory effect on phrenic motoneurons, as well as the inhibitory effect revealed by the delay of the next inspiration.

Observations such as these indicate that the superior laryngeal nerve fibers which excite inspiration are of a higher threshold than those which inhibit it. Therefore, in order to minimize complications due to simultaneous excitator and inhibitor effects, we have in some experiments employed the weakest stimuli which gave a sufficient degree of inhibition. This procedure, however, was not altogether satisfactory, since with weak stimuli the number of fibers stimulated could vary widely from time to time, due to changes in irritability. Fortunately, it proved possible to obtain relatively uncomplicated inhibitory effects from stronger stimuli provided the period of application was kept short. This is illustrated in figure 2, where 3 stimuli (*record B*) stopped the inspiratory discharge after only 2 impulses. This occurred in spite of the fact that these stimuli were strong enough to have not only an inhibitory, but also an excitant action, shown by prolongation of the activity when more shocks were applied in *record C*. In many of the experiments to be presented in this paper, the inhibitory effect was even stronger than in *record B* of figure 2, for the discharge often stopped after the first impulse. To accomplish this, higher frequencies of stimulation than those illustrated in the records of figures 1 and 2 were usually employed.

## RESULTS

### *Effects of Afferent Impulses During Inspiration*

*Afferent impulses early in inspiration.* Figure 3 shows an experiment in which various numbers of afferent impulses were sent in over one superior laryngeal nerve immediately after the first impulse discharged by a certain phrenic motoneurone. As few as 2 volleys of afferent impulses noticeably delayed the discharge of the second impulse in this motoneurone (*record B*). Progressively greater delays were caused as the number of afferent volleys was increased to 4 and to 9 (*record C* and *D*). After

this a remarkable change occurred when only one more was added to the number of afferent volleys. For with the change from 9 to 10, the interval between impulses was increased more than five-fold (*record E*). Compared to this large change, additional stimulation had but little additional effect (*F*).

The results of a number of similar experiments are shown graphically in figure 4, where the interval between the first and second impulses in a motoneurone discharge

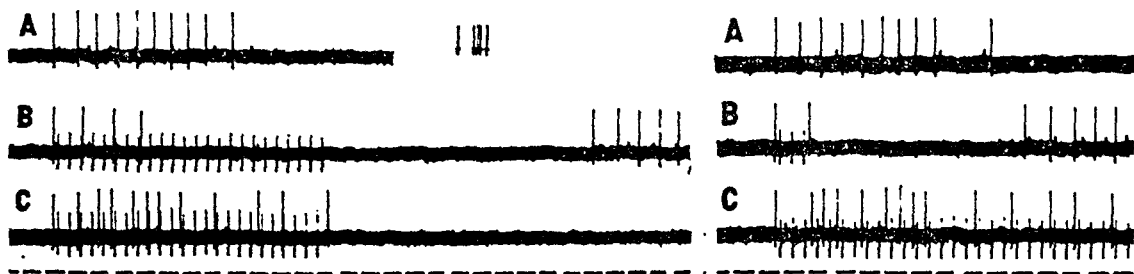


Fig. 1, left. EFFECT ON DISCHARGE OF IMPULSES from a motoneurone of the phrenic nerve, caused by stimulating one of the superior laryngeal nerves at two different intensities. Stimuli (indicated by regularly recurring artifacts) were weaker in *B* than in *C*. In the control record without stimulation (*A*), the arrows indicate the times at which the next inspiratory discharge started in this and in a number of other control observations. Time is marked at the bottom of this and all other reproductions of original records in  $\frac{1}{10}$ -second intervals.

Fig. 2, right. RESPONSE OF A PHRENIC MOTONEURONE to 3 stimuli (*B*) and to many stimuli (*C*), all of the same intensity, applied to one of the superior laryngeal nerves.

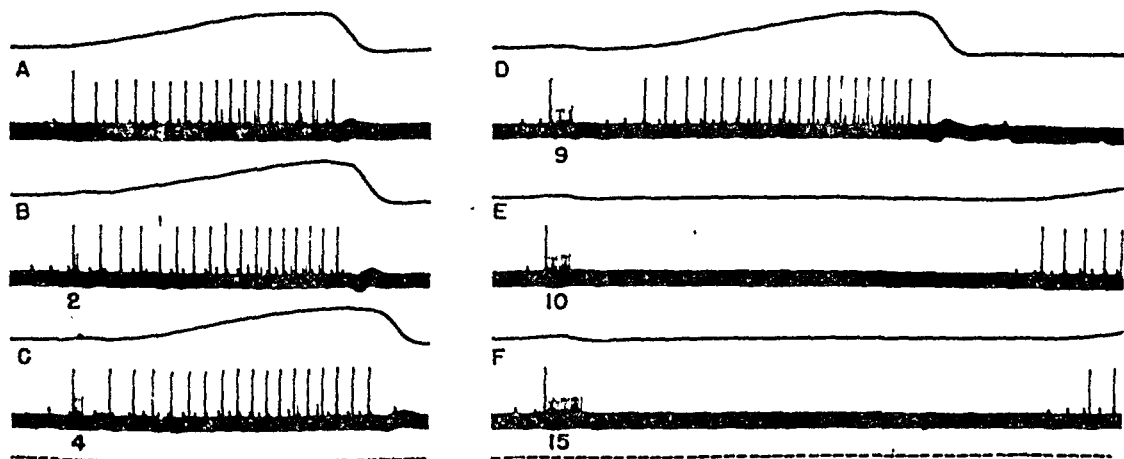


Fig. 3. CHANGES IN THE ACTIVITY OF A MOTONEURONE of the phrenic nerve, caused by the indicated numbers of volleys of afferent impulses over one of the superior laryngeal nerves. Vagus nerves cut. Normal duration of expiration at the time of these observations was 4.0 sec. The top line in these and many subsequent illustrations is a mechanical tracing of respiratory movements, with inspiration upward.

is plotted against the number of stimuli. In this graph the interval is expressed as percentage of the normal expiratory pause; this was measured by the average normal time between the last impulse in one inspiration and the first impulse in the next. There was in almost all experiments a certain number of afferent volleys which, when increased by one, resulted in a two- to seven-fold increase in the interval between impulses.

Thus there was usually a well defined transition, as the number of afferent impulses was increased, between a relatively short interruption of the motoneurone discharge and a relatively long interruption. It is interesting to attempt an explanation of this transition in terms of an hypothesis which has been advanced by others concerning the functional organization of the medullary respiratory centers (cf. 4, 7, 8). According to this hypothesis two groups of nerve cells in the medulla oblongata are concerned in respiration. One group, constituting the inspiratory center, discharges impulses during inspiration to the motoneurons supplying the inspiratory muscles. The other group of nerve cells form the expiratory center.

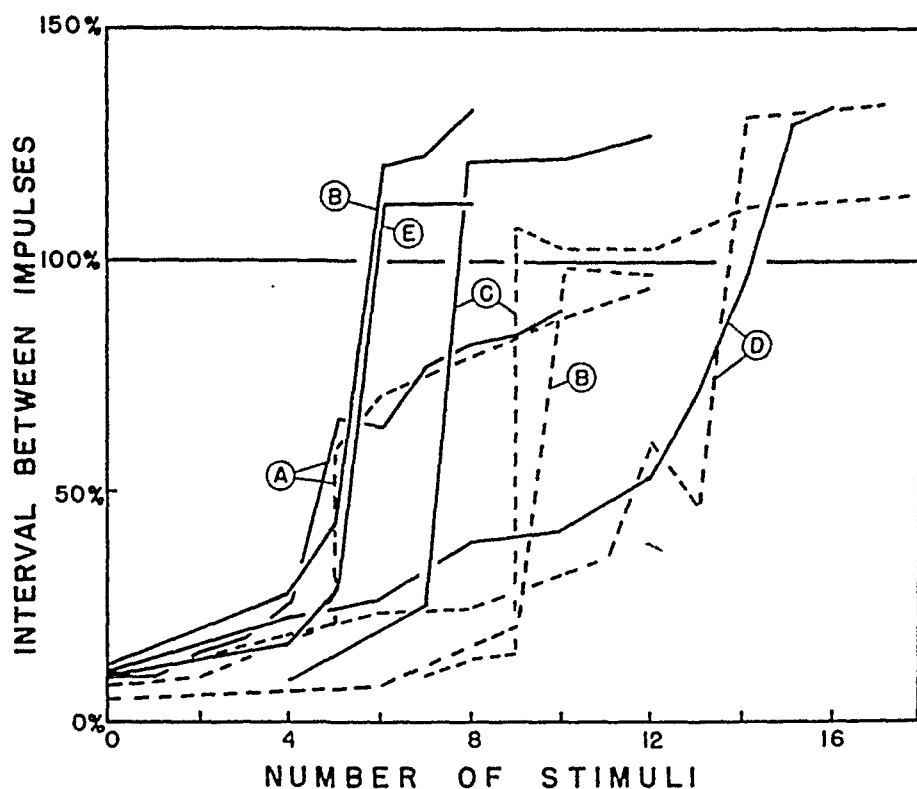


Fig. 4. INTERVAL BETWEEN FIRST AND SECOND IMPULSES in an inspiratory discharge from a phrenic motoneurone, as a function of the number of stimuli applied during the interval to one of the superior laryngeal nerves. Duration of interval is expressed as per cent of the normal duration of expiration. Observations on 5 different animals, indicated by letters. *Solid lines*, experiments with vagus nerves intact; *broken lines*, with these nerves cut. Stimulation at frequencies of 50 to 100/sec. The first impulse was discharged by the motoneurone under observation about 0.4 sec. after start of inspiration for *animal C*, earlier in inspiration for all others.

Each of these two groups is also supposed, when active, to exert an inhibitory effect on the other group, with the result that only one group can be active at a time.

In agreement with this hypothesis, we may suppose that small numbers of afferent impulses over a superior laryngeal nerve temporarily reduce but do not completely stop the activity of the inspiratory center. This reduction lowers the excitatory effect of the center on the phrenic motoneurons and at the same time reduces the inhibitory effect on the expiratory center, but not enough to permit the latter center to become spontaneously active. With larger numbers of afferent impulses, however, we may suppose that the inspiratory center is inhibited to such a degree that the

expiratory center is released and becomes active. Since this is a situation similar to that supposed to occur normally at the start of expiration, it is not surprising that in many animals the phrenic motoneurons remained quiet for a time comparable to the normal expiratory pause.

These considerations thus show that our observations are not in disagreement with the above hypothesis, which is at present generally thought to account for many of the known facts concerning the respiratory centers. Our observations might also be explained by other theories; but it does not seem profitable to introduce new lines of speculation until more direct methods have been devised for investigating the respiratory centers. Therefore, we merely wish to indicate here and elsewhere in this paper that certain findings are not in disagreement with the hypothesis referred to above.

In addition we wish to recognize another proposal which has often been coupled with the foregoing hypothesis, namely, that those afferent impulses which have an inhibitory effect on inspiration do so, not by a direct inhibitory action on the neurons of the inspiratory center, but indirectly through an excitatory effect on the neurons of the expiratory center. This assumption can also be reconciled with the above observations and with those to be presented later in this paper. Therefore, we will reserve for discussion at the end of this paper a consideration of whether the afferent impulses establish centrally an inhibitory or an excitatory state. Until then we will merely assume that the afferent impulses produce some undefined 'state' in the centers, which if sufficiently intense has the effect of stopping inspiration and starting expiration.

*Afferent impulses later in inspiration.* Effects similar to those described above for stimulation early in inspiration were also observed in some experiments where the afferent volleys were sent in later during inspiration. That is, a few stimuli delayed and sometimes slightly prolonged the remainder of the motoneuron discharge. As the number of afferent volleys was increased the delay progressively lengthened, until a critical number was reached at which all subsequent discharge was eliminated. These preparations thus exhibited the same abrupt transition of effect, when the number of afferent volleys was increased, as that described for stimulation early in inspiration (cf. fig. 8, *C* and *D*; also *curves C*, fig. 4).

In other experiments there was also a sharp transition with stimulation late in inspiration, but of a rather different sort (fig. 5). As before the principal effect observed with small numbers of afferent volleys was a momentary delay in the discharge of the next impulse (fig. 5 *B*). As the number of stimuli was increased, however, instead of reaching a condition in which *all* discharge subsequent to the stimulation was eliminated, the transition appeared as a dropping of many later impulses, but with 1 to 6 impulses still remaining immediately after the afferent volleys (fig. 5 *C*). The impulses discharged following stimulation are reflected in the figure by a 'bump' on the descending limb of the mechanical record of respiratory movements, indicating that a significant number of motoneurons took part in the final burst of activity. This burst of activity could be markedly decreased by applying a single additional stimulus, as shown by both the electrical and mechanical records in figure 5 *D*.

A similar discharge of a few impulses following stimulation was sought in a



number of experiments: no such discharge was ever recorded following stimulation in the first 0.4 seconds of inspiration (6 experiments); impulses appeared in nearly half the observations following stimulation 0.4 to 1 second after the start of inspiration (15 experiments); and the impulses always appeared when the stimulation was delayed more than 1 second (7 experiments). An illustrative example from a single experiment appears in figure 6. Thus the tendency for discharge of a few impulses following stimulation was found to increase progressively during inspiration. No significant alteration was caused in experiments where the vagus nerves were cut, except that tests could then be made later after the start of inspiration, since the latter was prolonged.

In one animal a still different manner of stopping inspiration was observed when afferent impulses were sent into the respiratory centers near the middle of inspiration. Records from this experiment (fig. 7) show that, when increasing numbers of afferent volleys were initiated following the 8th impulse from a certain motoneurone, the number of subsequent impulses was progressively reduced, from a normal of 9 (*record A*), to 6 in *B*, 4 in *C*, 2 in *D* and 1 in *E*. A further increase in number of stimuli (*record F*) did not prevent the discharge of this remaining impulse, although elimination of the 'bump' on the mechanical record (compare *records E* and *F*) indicated that the additional volley of afferent impulses had a marked effect on the activity of other motoneurons. The progressive shortening of inspiration observed in this animal stood in marked contrast to the usual effects, in which inspiration shortened suddenly as the number of afferent volleys was increased.

*Threshold for stopping inspiration.* In accordance with the observations described above, it was possible to define a threshold for stopping inspiration in terms of the minimum number of stimuli of given strength and frequency required to stop the discharge from phrenic motoneurons. When the discharge could be stopped with no impulses appearing after the stimulation (as in figs. 3 and 8), then the threshold was defined as the number of stimuli corresponding to the mid-point of the steepest portion of curves relating interval to number of stimuli (such as the curves in fig. 4). When a few impulses followed the minimum number of stimuli which stopped inspiration (as in figs. 5-7), then the mechanical records were examined to see whether the impulses discharged after stimulation were sufficient to cause a 'bump' on the descending limb, such as that appearing in figures 5 *C* and 7 *E*. When such an inflection occurred it always vanished when the number of stimuli was increased by one or two (figs. 5 *D* and 7 *F*), even though the particular motoneurone under observation sometimes still fired briefly after the stimulation. Under these circumstances the threshold for stopping inspiration was assumed to lie midway between the minimum number of impulses which prevented the 'bump' on the mechanical record and the maximum number which failed to do so.

With the threshold for stopping inspiration thus measured by the number of afferent volleys, it was possible to determine quantitatively how the threshold varied under altered conditions, provided the intensity of stimulation and hence the sizes of the afferent volleys were kept constant.

*Changes in threshold during inspiration.* More afferent volleys were required to stop inspiration at its beginning than when the afferent volleys were initiated later in

the cycle. This is illustrated in figure 8. In this experiment, 8 afferent volleys, immediately after the first impulse from a certain phrenic motoneurone, caused a

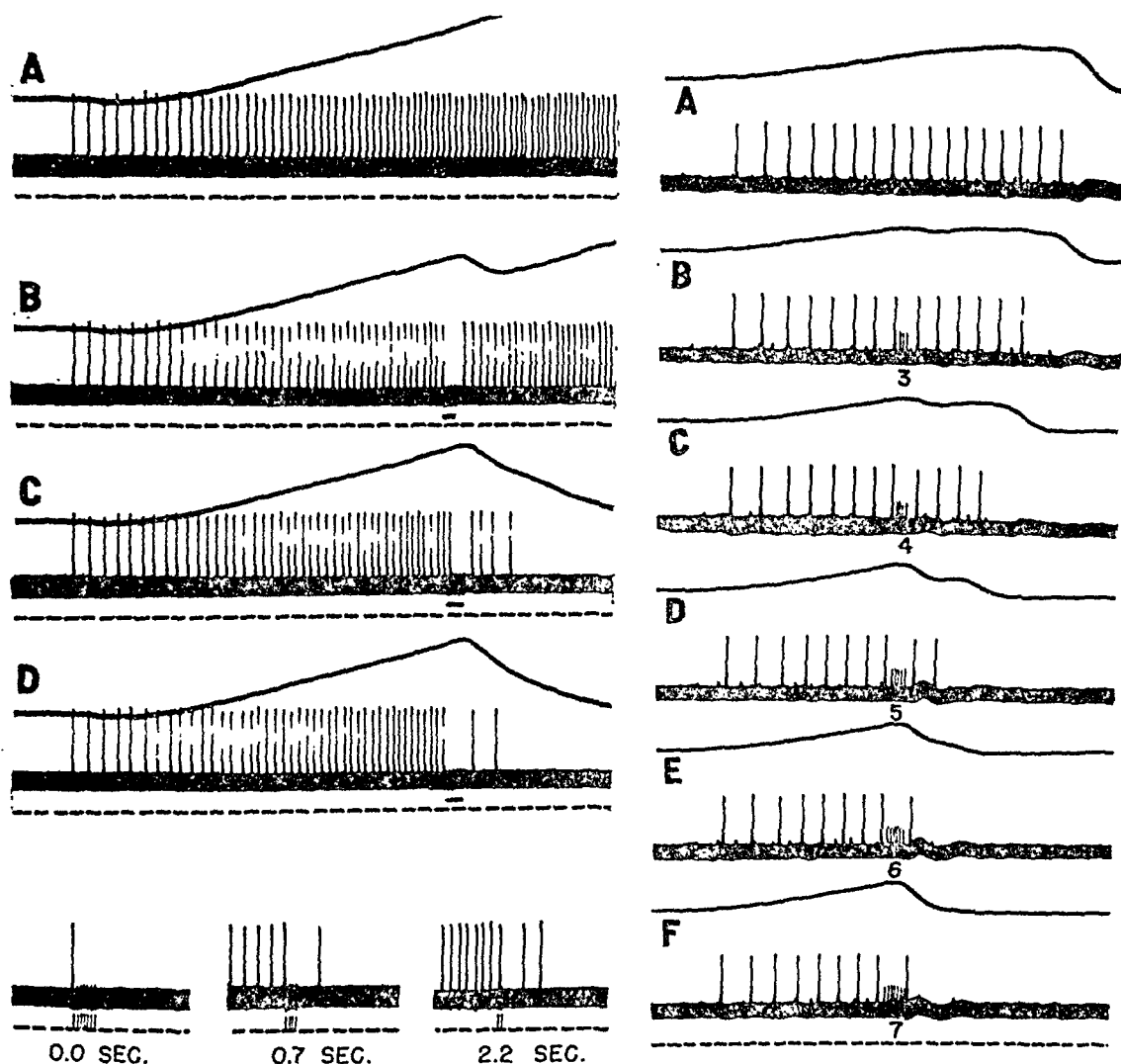


Fig. 5, upper left. EXAMPLE OF AN EFFECT ON PHRENIC MOTONEURONE DISCHARGE caused by stimulation late in inspiration. Following stimulation in *C* and *D* a few more impulses were discharged before activity ceased. Number of afferent volleys over one of the superior laryngeal nerves: 4 in *B*, 5 in *C* and 6 in *D*. Time of stimulation indicated by short horizontal line above timer. Vagus nerves cut. (Records taken while animal was breathing 5% CO<sub>2</sub>, 95% O<sub>2</sub>.)

Fig. 6, lower left. MANNER OF STOPPING PHRENIC MOTONEURONE DISCHARGE in a typical preparation. Minimum numbers of stimuli required to curtail activity were applied to one of the superior laryngeal nerves at indicated times after start of inspiration. The first (and only) impulse discharged appears in left record, but merely the end of activity is shown in the others. At the time these records were taken inspiration normally lasted more than 3 sec. Short vertical lines above time record indicate stimuli. Vagus nerves cut.

Fig. 7, right. UNUSUAL EFFECT OF AFFERENT IMPULSES over a superior laryngeal nerve on activity of a phrenic motoneurone. Discharge from the motoneurone was progressively shortened as number of afferent volleys was increased. Numerals indicate numbers of stimuli, which were applied at times revealed by artifacts. Vagus nerves cut.

moderate delay of the second impulse. Increasing the number of stimuli to 9 sufficed to stop activity for a time approximating normal expiration. When the afferent

volleys were sent in, not at the start of inspiration but about  $\frac{1}{2}$  second later, only 7 volleys rather than 9 were required to stop inspiration.

The change in threshold for stopping inspiration was demonstrated in another way in the experiment shown in figure 9. Here 5 stimuli, at a certain intensity and frequency, failed to stop the discharge from a phrenic motoneurone when applied early in inspiration (*records B and C*). When applied slightly later, similar stimuli did curtail the activity (*records D and E*). A similar decrease in threshold for stopping inspiration was observed by Boyd and Maaske (2) as stimuli to the vagus nerve were moved later in inspiration.

In several experiments the threshold for stopping inspiration was systematically determined at a number of times after the start of inspiration, with the results summarized graphically in figure 10 *A*. The progressive decline in threshold was clearly demonstrated in all experiments. Moreover, there was a surprising simplicity about

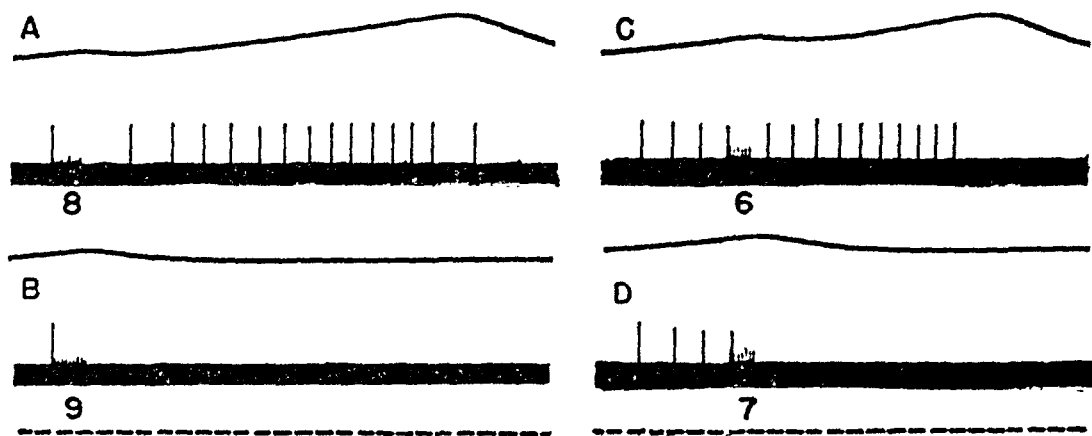


Fig. 8. NUMBER OF AFFERENT VOLLEYS over one of the superior laryngeal nerves required to stop discharge from a phrenic motoneurone was greater at start of inspiration (records on left) than later in the cycle (records on right). Numerals indicate number of stimuli, which were all of same strength. Vagus nerves cut.

this decline: in all experiments, within the accuracy of measurement, the threshold for stopping inspiration fell *linearly* during inspiration.

The several experiments have been further correlated in figure 10 *B*. In order to reduce all observations to a comparable time scale, the time from the start of inspiration to the last stimulus was expressed as percentage of the average duration of normal inspiration in each series of observations. The threshold number of impulses was also expressed in percentage by adjusting the scale of ordinates for each experiment so that the straight lines drawn in figure 10 *A* all passed through a threshold value of 100 per cent at the start of inspiration. It can be seen that, after these adjustments of coordinates, all the points fell near the single straight line drawn in figure 10 *B*. The fairly symmetrical scatter of points on the two sides of the line suggests that the deviations may be due to experimental errors of measurement and that the linear relationship may actually be followed very precisely.

The progressive decline in number of afferent impulses required to stop inspiration is evidence that changes in the respiratory centers, which eventually terminate inspiration, develop gradually throughout this phase of respiration. Further signifi-

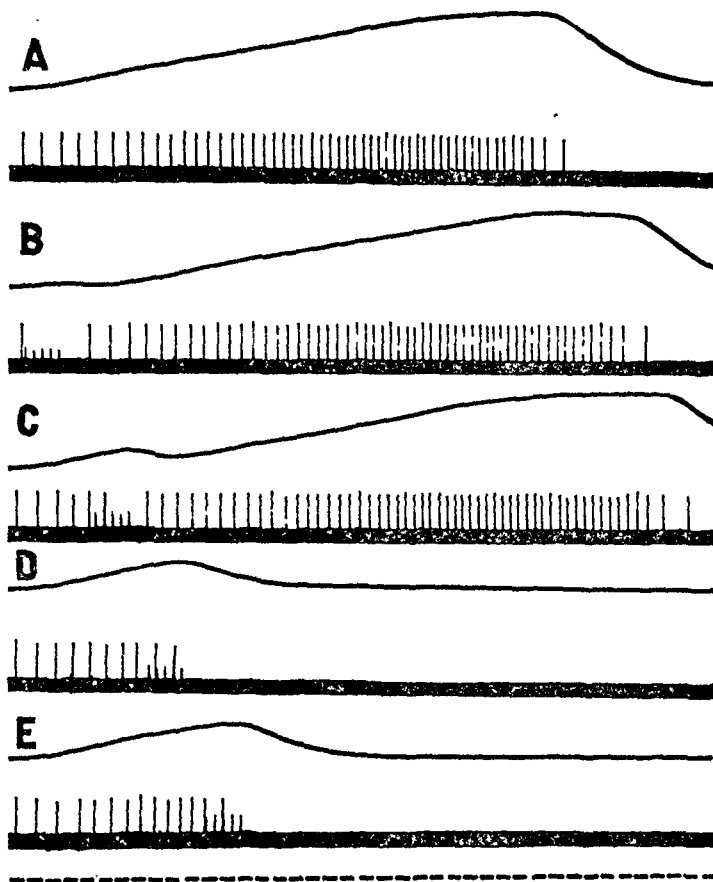


Fig. 9. WHEN 5 STIMULI OF SUITABLE STRENGTH were applied to one of the superior laryngeal nerves early in inspiration (*B* and *C*), they failed to stop the discharge from phrenic motoneurons. Later in the cycle similar afferent volleys cut inspiration short (*D* and *E*). Vagus nerves severed

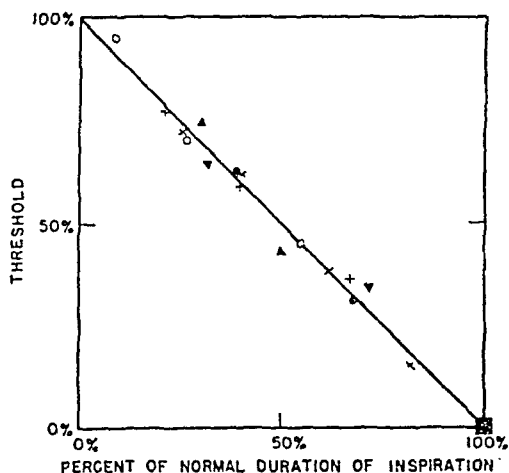
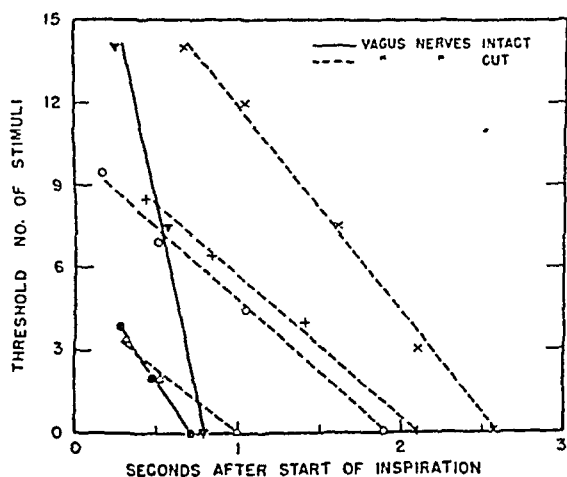


Fig. 10. THRESHOLD FOR STOPPING INSPIRATORY ACTIVITY by stimulating one of the superior laryngeal nerves at various phases of inspiration. Six series of observations on 5 different animals. Frequency of stimulation about 50/sec. in each. In *A* the number of afferent volleys required to stop inspiration is plotted against time from the start of inspiration to final stimulus. All lines drawn in this plot are straight. In *B* all straight lines of *A* have been made to coincide by suitable adjustments of scales of coordinates. See text for further explanation.

cance and the possible nature of these changes will be considered in the final section of this paper.

Quite puzzling is the constant rate at which the threshold declines, as indicated by the linear relationship shown in figure 10. Such a constant rate of change throughout the entire measurable course of a biological process is unusual. Possibly it is a result of the combination of several non-linear factors which add together to determine the measured change. It is, however, surprising that the several factors should by coincidence combine so as to give a linear result in each of several different preparations. Therefore, we suggest that this linearity may have a simple explanation and, when understood, will reveal an important detail concerning the mechanisms of the respiratory centers.

### *Effects of Afferent Impulses During Expiration*

The foregoing experiments offer direct evidence of progressive changes occurring in the respiratory centers during inspiration. Evidence of comparable progressive

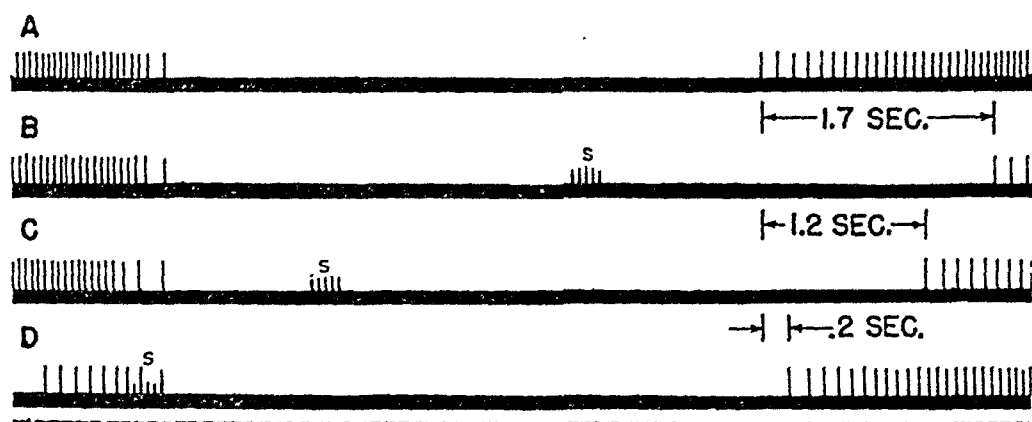


Fig. 11. LENGTHENING OF EXPIRATORY PAUSE, caused by a few volleys of afferent impulses over a superior laryngeal nerve. A is a control record without stimulation, showing end of one phrenic motoneurone discharge and start of the next. This record was carefully selected to show an expiratory pause of average duration. Five stimuli at fixed intensity were applied during expiration in B and C, during inspiration in D. Timing of stimuli indicated by stimulus artifacts appearing in each record at S. Vagus nerves cut.

changes occurring during expiration was obtained from interpretation of the effects next to be described. These effects were observed when afferent impulses were sent into the respiratory centers during expiration.

A few volleys of afferent impulses over a superior laryngeal nerve, during expiration, delayed the start of the next inspiration. This is illustrated in figure 11. A normal expiratory pause in the absence of stimulation is shown in record A. In record B, 5 stimuli were applied to the superior laryngeal nerve during the latter part of expiration, with the result that the next inspiration was delayed for 1.7 seconds. In record C of figure 11, the stimuli were applied earlier than in B, and the delay of inspiration was less. Even when the stimuli were further advanced so that they fell early in the preceding inspiration (record D), there was still a detectable prolongation of the expiratory pause. The progressive decrease in this prolongation of expiration, caused by moving the stimuli earlier in the respiratory cycle, is shown more completely by the data from two experiments plotted in figure 12. Here, as in so many

other observations in this paper, no obvious qualitative difference was caused by cutting the vagus nerves.

In interpreting the graphs of figure 12 one may assume, as we have done before, that the afferent impulses produce a certain altered 'state' within the respiratory center, which has the effect of inhibiting inspiratory activity. This state is progressively dissipated with the passage of time. Accordingly, the delay of the next inspiration is a quantitative measure of the residue of this state remaining at the time when inspiration would normally start. Moreover, the change in inspiratory delay with a given change in timing of the stimuli is an indication of the rate of decline of

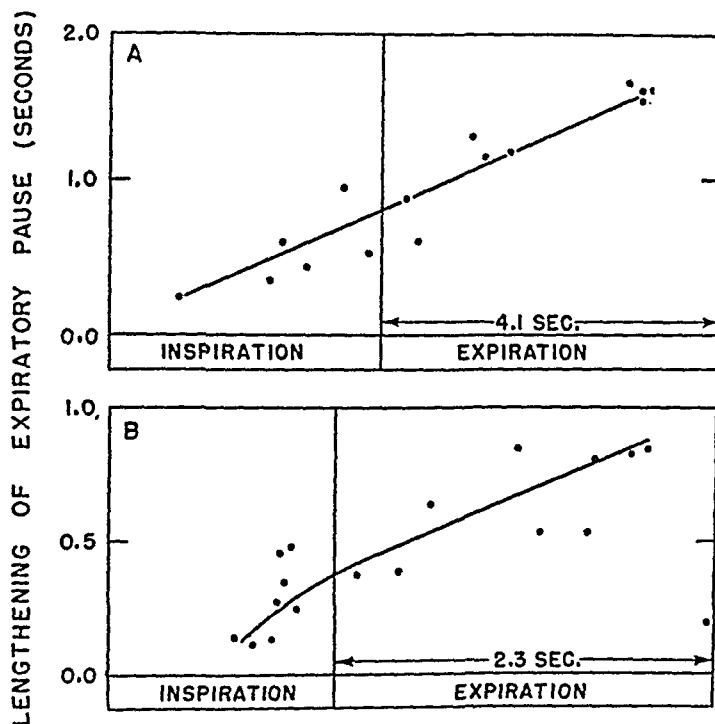


Fig. 12. INCREASE IN DURATION OF EXPIRATION (ordinates) caused by afferent volleys over a superior laryngeal nerve at various times during the respiratory cycle. Abscissae indicate times at which stimuli were applied. Lengthening of expiration was calculated by comparison with average of many normal expirations. Data from 2 different animals. Number, frequency and strength of stimuli were kept constant throughout the observations in each. Vagus nerves cut in *A*, intact in *B*. The numbers of stimuli were sufficient to stop inspiration when applied at the time of each point plotted during inspiration, but did not stop inspiration when initiated earlier.

the state. It is with this rate of decline that the remainder of this section is concerned.

In order to measure the rate of decline of the state produced by afferent impulses the delay of inspiration caused by afferent volleys at the end of expiration was compared with the smaller delay caused by similar volleys one second earlier. For this purpose the delays caused by stimulation at these two times were read from graphs such as those in figure 12, the effect of stimulation exactly at the end of expiration being determined by extrapolation. The percentage differences between the two delays varied considerably from animal to animal. It was discovered, however, that the differences were closely correlated with differences between the normal durations

of expiration in the several animals (fig. 13, *curve A*). The longer was the normal expiration, the less was the change in effect due to the one second change in timing of the afferent volleys.

The simplest interpretation of this observation is that the state, established centrally by the afferent impulses, declines most slowly in those animals with longest expirations. The significance of this finding will be discussed presently.

An even simpler relationship between the rate of decline of the central state and the length of expiration was suggested by comparing the delays of inspiration caused by equal numbers of afferent volleys sent in at the start and at the end of expiration.

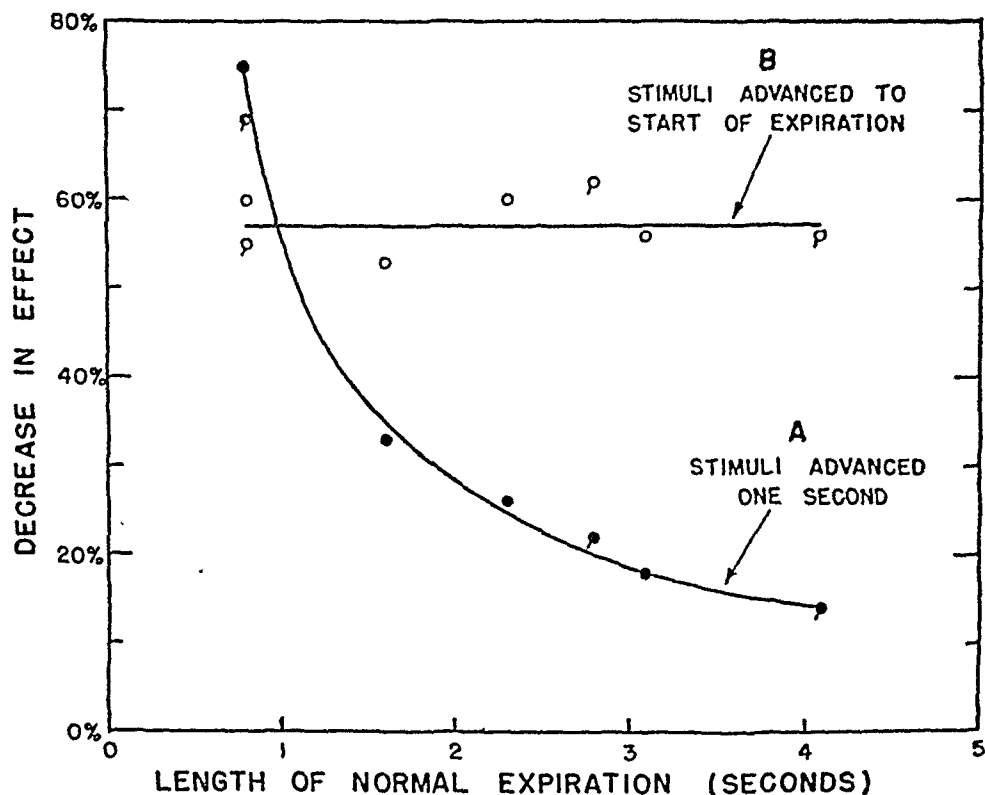


Fig. 13. DECREASE IN DELAY OF INSPIRATION when stimuli to one of the superior laryngeal nerves were moved from the end of expiration to the indicated earlier times. Tails drawn on some of the points identify experiments in which vagus nerves were cut.

In a manner similar to the preceding analysis, the percentage reduction in effect was calculated when the stimuli were advanced from the end to the start of expiration. This gave the very surprising result that the reduction in effect was about the same in all animals (fig. 13 *curve B*). In other words, if a certain number of afferent impulses delayed inspiration by a given amount when sent in at the end of expiration, then the same number of afferent impulses sent in at the start of expiration always produced a delay which was about 55 to 60 per cent shorter. It must be emphasized that in advancing the stimuli from the end to the start of expiration, the timing of the afferent volleys was in two cases changed by less than one second and in another by over four seconds, yet the percentage reduction of effect was about the same in

these, as in other animals with intermediate lengths of expiration. The simplest interpretation of this last observation is that the central state, produced by the afferent impulses, declined by about the same percentage in all animals during a time equal to the normal expiratory pause.

A possible explanation of the relationships just described can be given in terms of an hypothesis concerning the natural control of respiratory rhythmicity. Let it be assumed *a*) that in natural breathing a certain central state is developed progressively during inspiration, *b*) that inspiration stops when this state reaches a certain level and *c*) that inspiration starts again when this state has declined to a lower limiting level. Accordingly the length of the expiratory pause is determined by the time required for this state to fall from one critical level to another. Therefore, the longest expirations may be expected in those animals with slowest decline of the proposed naturally-occurring central state.

For simplicity let it further be assumed *d*) that the naturally-occurring central state is identical in nature with the state produced by afferent impulses. Accordingly the state produced experimentally by afferent nerve stimulation, like that which occurs naturally, should also decline most slowly in those animals with longest inspirations. This is in agreement with the findings expressed by *curve A* of figure 13.

To account for the uniform decrease in effect when the afferent impulses are moved from the end to the start of expiration (fig. 13, *curve B*), it is necessary to assume further *e*) that in natural breathing the two critical levels of the proposed state, at which inspiratory activity stops and starts, are about the same in all animals, or are at least in approximately a constant ratio to one another. Accordingly the percentage decline in the state during a time equal to one expiration would be the same for all animals. This is in agreement with the findings presented by *curve B* of figure 13.

### *Nature of the Central State*

Our observations have been found to agree with the concept that respiratory rhythmicity is brought about by the progressive development during inspiration of a central state, which stops inspiration when a critical level is reached and which subsides gradually during expiration. The progressive rise during inspiration is in agreement with the decrease in threshold for stopping inspiration by afferent impulses (fig. 10). The gradual subsidence during expiration is in agreement with the differences in effects produced by afferent impulses at different times during expiration and, particularly, with the relationship between these differences and the duration of normal expiration (fig. 13).

This concept of a waxing and waning central state is not new. It has been employed to explain how impulses, from pulmonary receptors and from the pneumotaxic center, influence the respiratory rate by exerting an inhibitory effect which increases progressively during inspiration. Certainly, the proposed state can be developed by factors other than afferent impulses from the lungs, for we have found that no qualitative change is caused by sectioning the vagus nerves.

Our observations not only furnish strong experimental support for this hypothesis that respiratory rhythmicity results from a fluctuating central state, but also



suggest a certain addition to it, namely, that the state declines slowly, even after the agent which develops it has ceased to act. This was shown by the long persistence of the inhibitory effect of a few volleys of afferent impulses during expiration. There is also evidence that the central state persists, at least for a short time, when it is

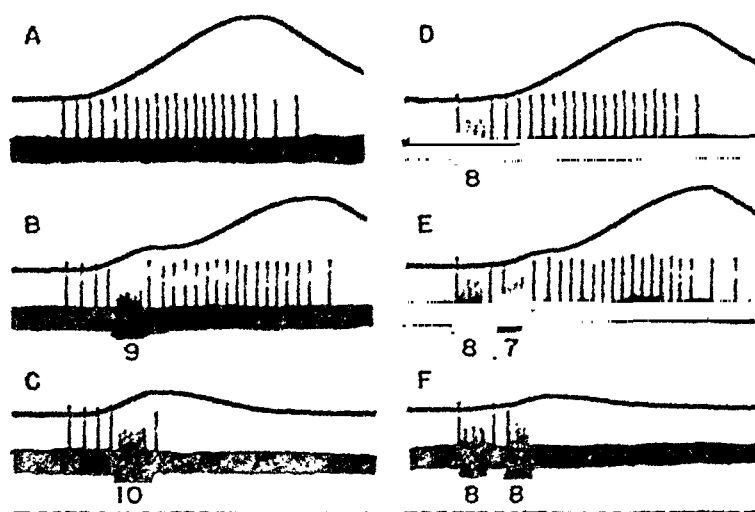


Fig. 14. REDUCTION OF THRESHOLD for stopping inspiration, caused by a few conditioning volleys of afferent impulses. Numerals indicate numbers of stimuli. Without conditioning, the threshold was between 9 volleys (B) and 10 (C). After 8 conditioning volleys (appearing alone in D), the threshold was between 7 (E) and 8 (F).

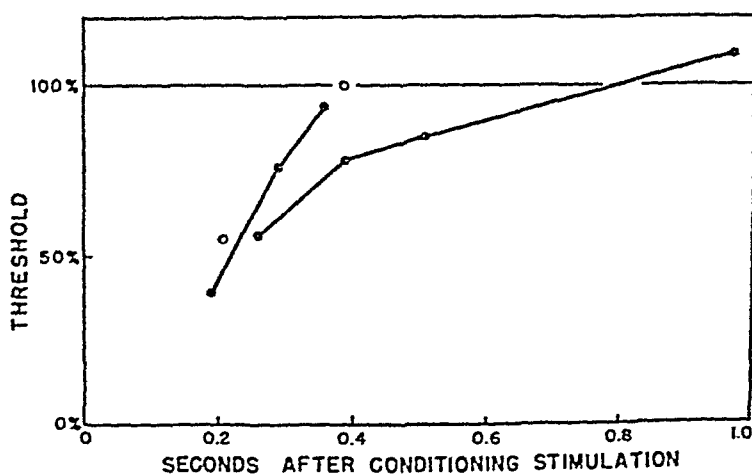


Fig. 15. REDUCTION OF THRESHOLD for stopping inspiration, following conditioning volleys of afferent impulses sent in at the start of inspiration. Experiments similar to that of fig. 14, the conditioning and testing stimuli being applied to one superior laryngeal nerve at a frequency of 50/sec. *Abscissae*: time from end of conditioning stimuli to end of testing stimuli; *ordinates*: threshold as percentage of control threshold found at the same time after the start of inspiration in the absence of conditioning stimulation. *Filled circles*, data from 2 animals with vagus nerves cut; *open circles*, another animal with vagus nerves intact.

produced by impulses sent in during inspiration. This has been revealed by the after-effects of impulses too few in number to stop inspiration. It was found that such volleys of impulses were followed by a reduction in threshold for stopping inspiration which lasted for at least several tenths of a second (figs. 14 and 15). A

similar enduring effect on threshold was found in one experiment when the conditioning impulses entered over one superior laryngeal nerve and the testing impulses over the other.

The nature of the proposed central state within the respiratory centers has not been determined. One possibility which must be considered is an inhibitory state at the inspiratory neurones in the medulla oblongata, which waxes during inspiration and wanes during expiration. This suggestion, however, encounters certain difficul-

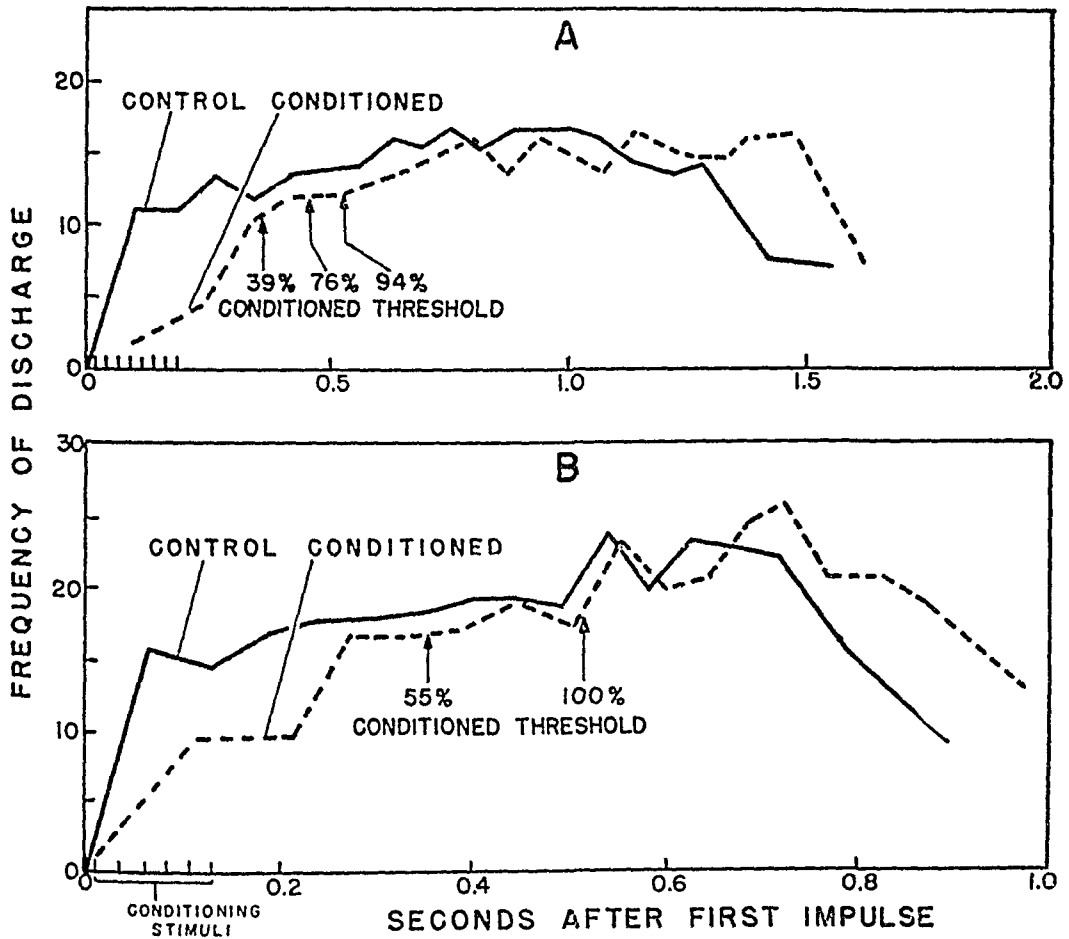


Fig. 16. FREQUENCY OF DISCHARGE from a phrenic motoneurone in a normal inspiration and during an inspiration modified by a few conditioning stimuli applied to a superior laryngeal nerve immediately after the first impulse in inspiration. Ordinates of curves are reciprocals of successive intervals between motoneurone impulses. *A* and *B*, for two preparations represented in fig. 14; *A* with the vagus nerves cut, *B* with these nerves intact. Thresholds for stopping inspiration following conditioning stimulation are indicated in percentage of normal threshold, at several times after the start of inspiration.

ties. In the first place one would expect that during natural breathing an incrementing inhibitory state would be reflected in a progressive slowing of the frequency of discharge from motoneurones supplying muscles of inspiration. On the contrary, it is known that during each inspiration there is a progressive increase in both the frequency of discharge from the individual motoneurones and in the number of motoneurones in action (3, 4). Therefore, it is suggested that the relevant fluctuating state be sought elsewhere than at the central inspiratory cells.

In agreement with this conclusion is evidence that the central state produced by stimulating an afferent nerve, which seems so closely related to the naturally fluctuating state, is also not associated with the inspiratory neurones in the medulla. Experiments already presented indicate that during inspiration the state produced by afferent impulses persists for several tenths of a second (figs. 14 and 15). Yet there was no corresponding persistent change in the frequency of discharge from a phrenic motoneurone. This is shown by the frequency analyses reproduced in figure 16. It is true that the frequency was slightly lower at the time of the tests after conditioning than it was at the same time without conditioning. But the frequency curves are approximately parallel at the time of the threshold tests: they do not approach each other as the threshold for stopping inspiration approaches normal. This lack of persisting effect on motoneurone discharge suggests a similar lack of persisting effect on the activity of cells of the inspiratory center, for Pitts (6), by stimulating the center while recording from a motoneurone, has revealed a close relationship between the activity of these two sets of cells. Consequently, it is unlikely that the persisting state produced by afferent impulses involves the inspiratory cells of the respiratory center.

Another possibility is that the naturally fluctuating central state, and the central state produced experimentally by afferent impulses, occur as excitatory changes at the expiratory cells of the medulla. Such an excitatory state could indirectly exert an inhibitory influence on the inspiratory cells, since the work of other investigators has already suggested the existence of appropriate neuronal connections within the medulla oblongata. This hypothesis concerning the central state does not encounter the difficulties found by assuming that the state affects the inspiratory cells directly, since a subthreshold excitatory state could develop at the expiratory neurones without influencing the activity of inspiratory cells. The subthreshold state could, however, facilitate the excitation of expiratory neurones by afferent impulses and thus account for the observed reductions in the threshold for stopping inspiration.

Finally it should be recognized that there are undoubtedly other possible locations for the fluctuating central state than those which have been discussed. Our experiments indicate that the state is not at the inspiratory neurones in the medulla. Obviously, further investigation is required to define as well as to locate this central state, which we have been forced to assume in order to account for the observations presented in this paper.

#### SUMMARY

1. Progressive changes in the respiratory centers, occurring during inspiration and during expiration, were revealed by quantitative variations in the effects of afferent impulses sent into the centers at various times during the respiratory cycle. The afferent impulses were initiated by electrical stimulation of the superior laryngeal nerves of anesthetized cats and the effects observed by recording the discharge of impulses from single phrenic motoneurons.

2. One or two volleys of afferent impulses, initiated immediately after the first

impulse discharged by a motoneurone at the start of inspiration, slightly delayed the discharge of the second impulse. As the number of afferent volleys was increased, the delay of the second impulse became progressively longer until a critical number of volleys was reached, at which the delay of the second impulse abruptly increased many-fold. This larger delay was usually similar in duration to the normal expiratory pause. When the afferent impulses were initiated later in inspiration, a sufficient number of volleys sometimes abruptly stopped the motoneurone activity, sometimes a few motor impulses were discharged following the afferent nerve stimulation before inspiration was brought to a premature close. The threshold number of afferent volleys required to stop inspiration declined progressively throughout the inspiratory phase of respiration. Threshold was found to be a linear function of time in each of the six preparations studied.

3. A few volleys of afferent impulses during expiration delayed the start of the next inspiration. Following a given number, frequency and intensity of stimuli, the delay of inspiration was less the earlier in expiration the afferent impulses were initiated. The delay was reduced more by a one-second advance in timing of the stimuli in animals with short expiratory pauses than in animals with longer pauses. On the other hand the delay of inspiration was about equally reduced by advancing the stimuli from the end to the start of expiration, in all preparations, regardless of the duration of expiration.

4. These findings can be explained by assuming that the respiratory center is influenced by an as-yet-undefined state somewhere within it, which is developed progressively during natural inspiration and subsides gradually during expiration. Activity of inspiratory neurones in the center is assumed to stop when this state attains a certain level and to resume when another, lower, level is reached. The effects of afferent impulses initiated by electrical stimulation may then be explained by assuming that these impulses cause additions to the naturally-occurring central state. Thus the progressive decline in threshold for stopping inspiration may be due to the gradual development of the proposed state during inspiration, so that a smaller contribution is required from the experimentally-initiated impulses in order to terminate inspiration.

The delay in start of inspiration, caused by afferent impulses during expiration, is explained by assuming a persistence of the central state developed by these impulses. Other evidence of such persistence was obtained by the finding of summation between the effects of two appropriately-spaced groups of afferent volleys. The relationships between the natural length of the expiratory pause and the rate of decline of the central state can also be explained.

5. It is possible that contributions to the naturally-occurring central state may normally be caused by afferent impulses from the lungs by way of the vagus nerves, but the state can also be developed by other factors, since no qualitative differences were found after sectioning the vagus nerves. During persistence of the central state developed by afferent impulses there was no corresponding reduction in frequency of discharge from phrenic motoneurones. This suggests that the central state is not an inhibitory state associated with the inspiratory cells in the respiratory centers. A possible explanation of the observed phenomena may, however, be

made by assuming that an excitatory state is developed at the central expiratory neurones.

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# SUDDEN VASOCONSTRICTION IN DENERVATED OR SYMPATHECTOMIZED PAWS EXPOSED TO COLD

JOHN F. PERKINS, JR.,<sup>1</sup> MAO-CHIH LI,<sup>2</sup> F. HOFFMAN<sup>3</sup> AND E. HOFFMANN

*From the Department of Physiology, Harvard University Medical School*

BOSTON, MASSACHUSETTS

WHEN an animal is placed in a cold room the skin of the extremities becomes cool owing to reflex vasoconstriction of the arteriovenous anastomoses (1). A sympathectomized or denervated extremity tends to remain warm because the vasoconstrictor pathways have been interrupted. This initially maximal vasodilatation does not, however, persist indefinitely. Starting a few hours after their nerves are severed, the blood vessels begin gradually to regain their 'tone' (2) and after a few days may constrict in cool surroundings (3). Such vasoconstriction after total denervation or sympathectomy has been described in man (4, 5), in monkeys (6) and in rabbits (3), but there is incomplete agreement with respect to the responsible mechanism (3-7).

It was noted by us (8) during the course of other studies in which a dog was placed in a room at 10°C., that the skin temperature of a sympathectomized paw, after remaining elevated for somewhat over an hour, suddenly fell sharply, and continued to decline until reaching essentially the same temperature as the opposite, normally innervated paw in which vasoconstriction was known to be nearly maximal. To our knowledge, an abrupt vasoconstriction occurring in sympathectomized or denervated extremities exposed to cold has not been reported previously, though a somewhat similar effect has recently been observed by Ungley (9) in cases of 'trench foot' of man, a condition in which degeneration of nerves occurs.

The present paper reports studies on the effects of prolonged cold on skin temperatures of sympathectomized or denervated vessels in the paws of cats and dogs. Sudden vasoconstriction appeared when the extremity was cooled below a certain 'critical temperature' and was complete enough to reduce blood flow to approximately one-tenth its former value. Reasons are given for attributing this reaction to the development of sensitivity to cold on the part of vascular smooth muscle after sympathectomy and total denervation.

## METHODS

Observations were made *a*) repeatedly on one unanesthetized, trained dog (Series I), *b*) on 7 lightly anesthetized cats while the whole body and the paws were exposed to cold (Series II) and *c*) on 9 lightly anesthetized cats while the body was kept warm and the paws only were exposed locally to cold (Series III).

In those animals, as indicated in the tables, in which sympathectomy was performed, the lumbar sympathetic chain with its ganglia was removed from the level of the renal artery to the bifurca-

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Received for publication September 17, 1948.

<sup>1</sup> Present address, Dept. of Physiology, University of Chicago.

<sup>2</sup> Fellow of the American Bureau for Medical Aid to China.

<sup>3</sup> Rockefeller Traveling Fellow (Santiago, Chile).

tion of the aorta, thereby providing an essentially preganglionic sympathectomy of the lower hind limb. In another group of animals the sciatic nerve was sectioned close to its point of emergence from the sciatic notch and proximal to all branches of the nerve. In a third group of animals, more extensive procedures aimed at total denervation of the leg were carried out as described below.

At periods ranging from 0 to 130 days after operation the effect on skin temperature of prolonged exposure to a cold environment was tested. The trained anesthetized dog was supported by slings in a wheeled Pavlov stand. Anesthetized cats, given 22.0 to 32.5 mg. of pentobarbital (Nembutal, veterinary) intraperitoneally, a dose which did not abolish shivering or reflex vasoconstriction, rested with their ventral surface on a wooden stand so that the paws hung freely in the air.

Skin temperatures were measured on the dorsal surfaces of the paws at the base of the digits by iron-constantan thermal junctions applied under loose adhesive tape or by collodion and a wisp of cotton. The reference junctions were immersed in oil in a thermos flask, or in boiling ether with a reflux condenser, each thermocouple circuit being led independently to a galvanometer through a selector switch so that readings could be made rapidly in rotation, free of complicating skin potentials. Repeated calibrations indicated that the measurements were accurate to within  $\pm 0.5$  degrees C. Rectal temperature was determined by a mercury thermometer left continuously in place or by means of a thermocouple.

Each experiment was started with maximal dilatation of the cutaneous vessels of the paws by placing the animal in a thermoregulated room at  $30^{\circ}\text{C.} \pm 0.5^{\circ}\text{C.}$  In some instances one or two electrical heating pads were placed around the body to hasten the abolition of vasoconstrictor tone. When vasodilatation was complete, as indicated by skin temperatures consistently between  $34$  and  $36.5^{\circ}\text{C.}$ , the animals were taken into another thermoregulated room set at the desired temperature between  $25^{\circ}$  and  $0^{\circ}\text{C} \pm 1.0^{\circ}\text{C.}$  Skin temperatures were measured at intervals of five minutes or less.

For localized cooling of the paws alone and to provide more rapid changes in the temperature of the air surrounding the paws, a  $30 \times 20 \times 12$  inch box was lined with copper pipes connected to a source of brine at  $-13^{\circ}\text{C.}$  A fan circulated air at low velocity through the narrow space between an inner sheet metal lining, on three sides of the box, and the brine pipes on the walls of the box. The desired constant temperature was obtained by means of a thermostatically controlled electric heater used in conjunction with manual regulation of the flow of brine. The cat was supported above the box by a sling, its paws projecting into the box through holes in a felt diaphragm in the cover. The box was located in one of the thermoregulated rooms so that the animal's body could be surrounded by air at any desired temperature, whereas the paws, or the entire legs if desired, could be surrounded by air at any other temperature. In most cases, the room was kept at  $30^{\circ}\text{C.}$  and the box, initially at  $30^{\circ}\text{C.}$ , was gradually cooled by steps to temperatures sometimes as low as  $-5^{\circ}\text{C.}$  Skin, rectal and box temperatures were measured thermoelectrically.

Changes of skin temperature were translated into approximate changes of blood flow by means of Burton's 'Thermal Conductivity Index,' ( $\text{TCI}' = (T_{\text{skin}} - T_{\text{air}})/(T_{\text{rectum}} - T_{\text{skin}})$  (in which ratio ' $T$ ' stands for temperature). The declining portions of the skin temperature curves were also plotted semi-logarithmically to determine the abruptness and degree of constriction by *a*) the straightness of the line and *b*) the thermal time constant ' $k$ ' as will be described more fully below.

### *I. General Characteristics of the Sudden Vasoconstriction*

Figure 1, (*upper*) shows in the unanesthetized dog the usually described response observed during cooling after lumbar sympathectomy. In the warm room kept at  $30^{\circ}\text{C.}$ , skin temperatures were  $34^{\circ}\text{C.}$  or more. When the dog was exposed to air at  $15^{\circ}\text{C.}$ , the skin temperature of the normal extremities approached environmental temperature as vasoconstriction developed, but the temperature of the sympathectomized extremity remained relatively constant at  $26$  to  $27^{\circ}\text{C.}$  When the dog was exposed to a temperature of  $10^{\circ}\text{C.}$ , (fig. 1, *lower*) the sympathectomized extremity remained relatively warm at first, but when its temperature reached  $24^{\circ}\text{C.}$ , it began, as a result of sudden vasoconstriction, to cool abruptly, producing a distinct 'angle'

in the plotted temperature curve. Thereafter, the temperature of the sympathectomized paw declined along a curve similar in shape and time relations to the skin temperature curve of the vasoconstricted, normally innervated opposite paw.

In these and other similar experiments, throughout a considerable period prior to the sudden vasoconstriction, there was often a gradual decline in the skin temperature of the sympathectomized paw, indicating gradual vasoconstriction. In addition, fluctuations in the skin temperature of one to two degrees often appeared just before

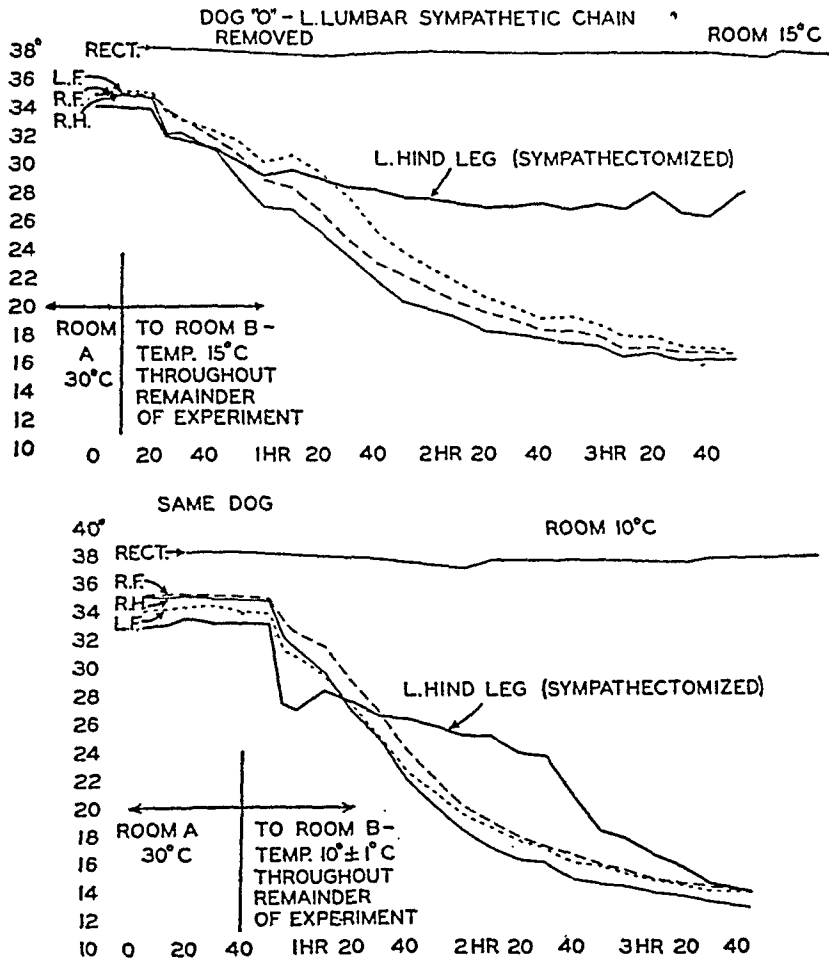


Fig. 1. TEMPERATURE CHARTS, in °C., from two experiments performed on different days on an unanesthetized dog in a cold room. Skin temperatures in these and subsequent experiments were determined on dorsal aspect of paws. *Upper chart.* With room at 15°C. vasodilatation persisted. *Lower chart.* With room at 10°C. sudden vasoconstriction, indicated by a sudden fall in skin temperature, occurred in the sympathectomized paw.

or after the sudden vasoconstriction. However, once the temperature of the sympathectomized paw had abruptly declined more than two or three degrees, in most experiments it did not rise again to its previous level, but continued to fall.

Following the sudden vasoconstriction and after the temperature of the paw had leveled off at just above environmental temperature, sudden vasodilatation, indicated by a sudden rise in skin temperature, was observed in three experiments (two on animal 30, table 3) during gradual warming of the air surrounding the paw (fig. 4).



## II. Evidence for a 'Critical Temperature' at Which Sudden Vasoconstriction Occurred

The three experiments charted in figures 1 and 2 provide data indicating that the sudden vasoconstriction did not occur until the skin of the sympathectomized paw was cooled to a certain 'critical' temperature. Thus, with the room at  $15^{\circ}\text{C}.$ , (fig. 1, *upper*) the gradient of temperature between the body (rectal temperature) and the room was such as to cool the vasodilated paw to somewhat above  $26^{\circ}\text{C}.$ , but the temperature of the paw never fell below that value and the skin of the paw remained vasodilated. However, with the room at  $10^{\circ}\text{C}.$ , (fig. 1, *lower*) the correspondingly greater cooling gradient sufficed to lower the skin temperature of the vasodilated paw to approximately  $24^{\circ}\text{C}.$ , at which temperature sudden vasoconstriction occurred.

Further evidence for the existence of a critical temperature for vasoconstriction is provided by the experiment charted in figure 2, performed after both lumbar sympathetic chains had been removed from the dog. Sudden vasoconstriction occurred in the right hind paw after three hours in the cold room, but vasodilatation

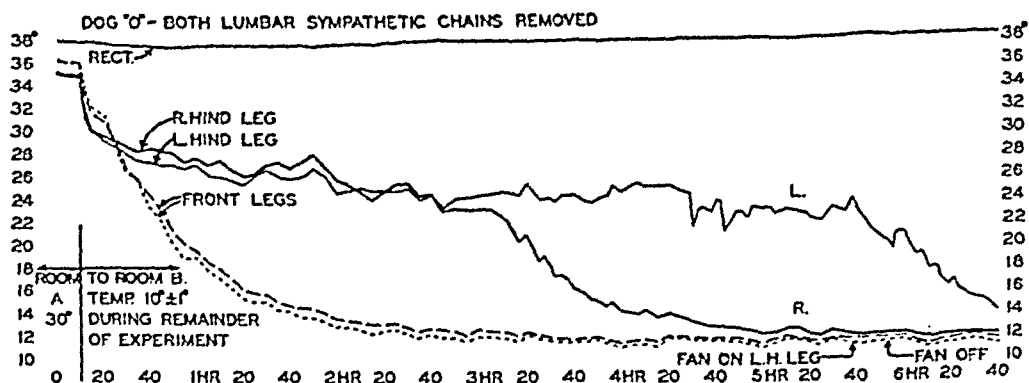


Fig. 2. FOLLOWING REMOVAL OF BOTH LUMBAR SYMPATHETIC CHAINS, sudden vasoconstriction occurred in right hind paw after three hours in the cold room, but did not occur in left hind paw until it was cooled for a brief period by an electric fan.

was still present in the left hind paw, apparently less 'sensitive' to cold, even after five hours in the cold room. At this point, the left hind paw and leg were cooled for 15 minutes by means of an electric fan, following which sudden vasoconstriction occurred.

That it was the skin temperature of the operated paw, rather than the room or rectal temperature, which determined whether or not sudden vasoconstriction occurred is indicated by reference to the columns entitled 'Temperatures at Time of Sudden Vasoconstriction', in tables 1, 2 and 3. These temperatures were taken in each experiment at the time of the 'angle' in the skin temperature graph. It will be noted that the absolute values of room, box or rectal temperatures bear no consistent relation to the sudden vasoconstriction except insofar as they together constitute a gradient of temperature capable of cooling the operated paw. In the experiments included in the tables, this gradient was adjusted so as to cool the paw gradually to its critical temperature. Such experiments were characterized by the usual vasodilatation of sympathectomy or denervation, which persisted, as indicated by an elevated though very gradually declining portion of the skin temperature graph,

until the sudden vasoconstriction occurred. In those experiments, not included in the tables, performed at low temperatures, e.g.  $0^{\circ}$  to  $5^{\circ}\text{C}$ ., the graph of skin temperature for the operated paw closely resembled the graphs for the normal paws. Thus, vasoconstriction occurred promptly in the operated as well as in the normal paws, apparently because the operated paw was cooled below its critical temperature very soon after the animal entered the cold room. In most experiments the time of onset of shivering was noted and the intensity was graded on an arbitrary scale of 0 to 5. There was found to be no correlation between the time of onset or severity of shivering and the time of sudden vasoconstriction.

TABLE I. SERIES I. UNANESTHETIZED DOG—COLD ROOM

ANIMAL NO.	OPERATIVE PROCEDURE	DAYS POST-OP.	TEMP. AT TIME OF SUDDEN VASOCONS., $^{\circ}\text{C}$ .			THERMAL CONDUCTIVITY INDEX		STRAIGHTNESS OF SEMI-LOG PLOT	TIME CONSTANT k, MIN.	
			Room	Rectal	Skin, paw	Before vaso-cons.	After vaso-cons.		Normal paw	Op. paw
Dog o	Removal l. lumbar symp. chain	12	$10^{\circ}$	$39.6^{\circ}$	$23.8^{\circ}$	0.89	0.12	++++	50	48
"	Removal rt. lumbar symp. chain 9 mos. later	10	$10^{\circ}$	$37.9^{\circ}$	L. $20-26^{\circ}$	1.4	0.12	++	46	33
					R. $21-23^{\circ}$	0.9	0.12	++		36
"	Same	12	$15^{\circ}$	$37.8^{\circ}$	L. R.?(fan used)	1.7 1.65	0.10	++++	48	45
"	Same	24	$10^{\circ}$	$38.0^{\circ}$	L.?(fan used) R. $22.4-23.2^{\circ}$	0.80 0.87	0.08	++ ++++	41	42 32
"	Same	130	$5-3^{\circ}$	$37.4^{\circ}$	L. $20^{\circ}$ R. $20-22^{\circ}$	1.10 1.40	0.02	+++	44	
Averages.....					$22.1^{\circ}$	1.19	0.12		45.8	39.3

Certain exceptions to the sudden vasoconstriction should be noted. First, the skin temperature did not always fall abruptly enough to produce a distinct 'angle', but in roughly one quarter of the experiments fell with increasing rapidity over a few degrees (indicated by a range of temperatures in the tables). Second, in six experiments (not included in the tables) there was a continuously gradual decline in skin temperature, rather than a sudden fall. This latter was found to be attributable in all but two of these experiments to the type of operation performed, or to the interval after operation, as described below.

### III. Degree of Vasoconstriction and Relative Decrease in Blood Flow

1) *Burton's Thermal Conductivity Index.* Burton's (10) 'Thermal Conductivity Index' ('TCI') consists of the following ratio:  $(T_{\text{skin}} - T_{\text{air}})/(T_{\text{rectum}} - T_{\text{skin}})$

which provides an approximate measure of peripheral blood flow provided the skin temperature has reached a steady state, having leveled off under conditions of relatively constant blood flow, rectal and air temperatures. As indicated in the tables, TCI of the operated paw was calculated for each experiment at the 'angle' in the skin temperature graph (before vasoconstriction) and again after the skin temperature had leveled off at its lowest value (after vasoconstriction). Comparison of averages

TABLE 2. SERIES II. CATS LIGHTLY ANESTHETIZED WITH NEMBUTAL—COLD ROOM

ANIMAL NO.	OPERATIVE PROCEDURE	DAYS POST-OP.	TEMP. AT TIME OF SUDDEN VASOCONS., °C.			THERMAL CONDUCTIVITY INDEX		STRAIGHTNESS OF SEMI-LOG PLOT	TIME CONSTANT k, MIN.	
			Room	Rectal	Skin, paw	Before vaso-cons.	After vaso-cons.		Normal paw	Op. paw.
3	Section sciatic	0.2	5°	Paw cooled to 23° without vasoconstriction						
		2	5°	33°	?26° (fan used)	2.5			23	
		5	17°	39.8°	29.6°	1.1	0.05	++++		24
		7	18°	37.4°	28°	1.0		+++	21	22
15	Section sciatic	2	4-2°	30.4°	19°	1.2			22	28
		4	5°	38°	29.1	2.7				
		6	13°	37.8°	27°					
		9	20-18°	37.8°	30.7°	1.3	0.22	+++		19
17	Bilat. lumbar sympathectomy & denerv. adrenals	7	11°	32.8°	L. 23 R. 22	0.93 1.5	0.10 0.10	++++ +++	19 20	19 25
17	"	38	16°	31.5°	L. 22-23 R. 23	1.0 1.2	0.11 0.16	++++ ++	22 22	23 33
9	Section sciatic Adrenalectomy	4 0	21°	39°	29.7°	0.96	0.07	++		30
21	Total denerv. leg Adrenalectomy	3 0	17°	37.7°	26-27	1.1	0.16	++++	21	17
18	Total denerv. leg	5	14°	32.2	25-27	2.0	0.12	+++	16	32
20	Total denerv. leg	3	16°	39°	22-26	0.77	0.05	+++	23	24
Averages.....						1.38	0.11		20.9	24.7

of these two values indicates that the blood flow decreased by a factor of 9.9 in the dog and 10.5 in the cats (fig. 3, lower).

2) *Semi-logarithmic plots of declining portions of skin temperature curves.* a) *Straightness of plotted curve.* When the declining portions of skin temperature curves such as those in figures 1 and 2 were plotted semi-logarithmically, the resulting curves were found in most cases to be straight lines, which indicates that the original skin temperature graph was an exponential curve of the form  $(T - T_0) = e^{-t/k}$  where  $T$  = skin temperature,  $T_0$  = minimal skin temp.,  $t$  = time, and  $k$  is the 'time con-

TABLE 3. SERIES III. CATS LIGHTLY ANESTHETIZED WITH NEMBUTAL—PAWS IN COLD BOX

ANIMAL NO.	OPERATIVE PROCEDURE	DAYS POST-OP.	TEMP. AT TIME OF SUDDEN VASOCONS., °C.			THERMAL CONDUCTIVITY INDEX		STRAIGHTNESS OF SEMI-LOG PLOT	TIME CONSTANT k, MIN.	
			Room Box	Rectal	Skin, paw	Before vaso-cons.	After vaso-cons.		Normal paw	Op. paw
17	Bilat. lumbar sympathect. and denerv. adrenals	52	29° 17°	38°	L. 26-29° R. 29-31°	1.2 2.3	0.12 0.25	++	71 17	17
24	L. lumbar sympathect., r. adrenal denerv., l. removed	14	24° 17°	38°	30.4° rose at 20.4°	1.5	0.27	++	26	20
27	L. lumbar sympathect., r. adrenal denerv., l. removed	11	30° 15°	38.6°	28-29°	1.65	0.03	++	24	20
23	Leg denervated Adrenalectomy	7 0	32° 0°	41.5°	22.3-24°	1.4	0.16	+++		16
28	Total denervation leg, including section of skin of thigh	6	31° 15°	36°	27.8°	2.1	0.13	+++	16	17
30	Leg denervated Leg amputated, except art. & vein	4 0	34° 9°	39.2°	26.2° rose 22.6-24.6°	1.2	0.19	++++	16	17
25	Section sciatic <sup>1</sup>	14	30° 0.5°	41.5°	23-26°	1.7	0.08			
25	"	7	30.5° 14°	34°	26°	2.2	0.18	+++	20	18
25	"	21	30° 11°	34.5°	23.5°	1.1	0.16	+++	23	15
24	Complete section of brachial plexus	14	23° 1°	37°	28-29° rose at 19°	3.1	0.4			
26	Section sciatic	3	32° 1°	39.5°	20	0.93	0.27	++		21
22	"	4	32° 2°	37°	25-27°	2.6	0.32	+++		17
Averages.....						1.78	0.19		20	17.8

<sup>1</sup> The sciatic nerve was sectioned following evidence for regrowth of the one lumbar sympathetic chain which had been removed previously.

stant' of the curve. Figure 3, *upper right*, shows one of these plots from an experiment on a cat with a denervated leg. As indicated in the tables, the straightness of each curve was graded on an arbitrary scale of 0 to 4 plus.

Newton's 'Empirical Law of Cooling' predicts that the temperature of a given warm body with constant thermal conductivity will decline along a curve having an exponential equation of this type. Conversely, if a declining temperature curve is found to have an equation of this type, the thermal conductivity of the corresponding warm body must be constant. In the paw this indicates that the blood flow was

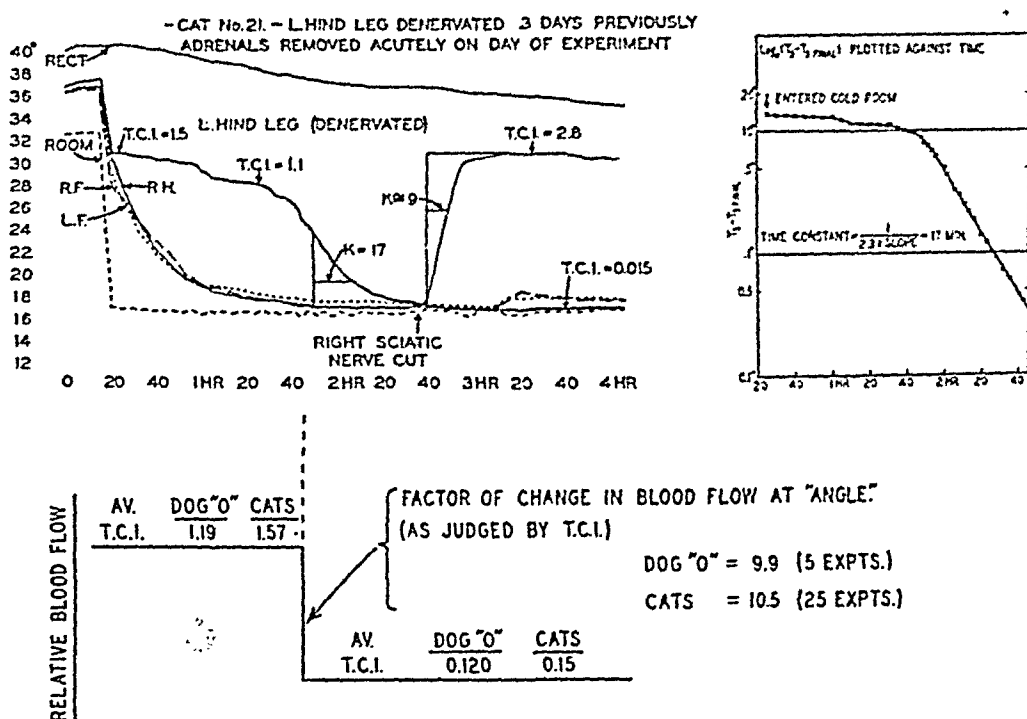


Fig. 3. *Upper left* shows sudden vasoconstriction after adrenalectomy; also illustrates method of determining, by direct measurement on the curve, the time constant 'k' (an indirect measure of relative blood flow) of an exponentially declining, or rising skin temperature curve. Thermal Conductivity Indices were calculated at points shown. *Upper right* illustrates straightness of semi-log plot of declining skin temperature curve, and more accurate method of determining *k* from slope of semi-log plot. *Lower* summarizes results of analyses of skin temperature data from 5 experiments on a dog, and 25 experiments on cats. Coincident with the 'angle' in a skin temperature graph, as in the upper left of this figure, there was, as a result of sudden vasoconstriction, a sudden decrease in relative blood flow, to an average value of approximately  $\frac{1}{10}$  the previous value.

constant throughout the declining portion of the curve, because the thermal conductivity of the paw is made up of *a*) the thermal conductivity of the dead tissues (constant) plus *b*) the blood flow.

Therefore, the decrease in blood flow, which fell to approximately one tenth of its previous value, as measured by the thermal conductivity index, occurred suddenly at the time of the 'angle' in the skin temperature graph, and no further decrease occurred thereafter. These facts are summarized diagrammatically in figure 3, *lower*.

*b) Thermal time constant 'k'.* The reciprocal of the slope of the semi-logarithmic

plot gives the thermal time constant, ' $k$ ' in minutes, which may also be determined, though less accurately, by measuring on the original skin temperature graph the time required for the curve (if on inspection it appears to be an exponential) to decline to 36.8 per cent of its original height, or to rise to 63.2 per cent of its final height, as shown in figure 3, *upper left*. From mathematical considerations, Burton (11) has shown that there should be a linear relation between  $1/k$  and the Thermal Conductivity Index. Thus, in a given paw  $k$ , as well as TCI, provides an approximate measure of relative blood flow, a short  $k$  corresponding to a rapid flow.

As shown in the tables, the values of  $k$  for the operated paws are of the same order of magnitude as for the normal paws, which indicates that vasoconstriction as a result of cold reduces the blood flow in the operated paw by approximately the same amount as reflex vasoconstriction does in the normal. That the blood flow was extremely small in either case is shown by the fact that when animals were killed while their paws were vasodilated, the values of the resulting  $k$ 's were not significantly greater than for the vasoconstricted paws of living animals.

#### *IV. Rise in Critical Temperature with Increasing Intervals after Denervation*

Reference to table 1 indicates the relative constancy of the critical temperature for the sympathectomized paw or paws of the dog, the individual values varying by no more than  $\pm 2^{\circ}\text{C}$ . from the average value of  $22.1^{\circ}\text{C}$ . in all but one out of six values, during a period of from 10 to 130 days after lumbar sympathectomy. In view of the progressive 'regain of tone' known to occur in blood vessels during the first few hours or days after they have been deprived of their sympathetic innervation, it was decided to determine whether there was a day-by-day rise in the value of the critical temperature, indicating increasing 'sensitivity' to cold during the period immediately after operation.

Though they were attempted earlier, successful experiments on a dog less than 7 to 10 days after lumbar sympathectomy were found to be impossible owing to the period required for convalescence. It was therefore decided to section the sciatic nerve, containing the post-ganglionic sympathetic nerves to the paw, an operation so trivial as to require no period of convalescence. As shown in table 2, a series of experiments was performed on each of 2 cats (no. 3 and 15) at varying intervals after sectioning the nerve. The results indicate that there was a definite tendency for the critical temperature to rise during the first few days after this operation. Thus, five hours after cutting the sciatic nerve in *animal 3*, there was no sudden vasoconstriction, the paw remaining vasodilated even though it was cooled to  $23^{\circ}\text{C}$ ., by reducing the temperature of the room to  $5^{\circ}\text{C}$ . However, two days after operation, sudden vasoconstriction occurred when the skin was slightly below  $26^{\circ}\text{C}$ ., the exact value being obscured by the brief use of a fan to cool the paw. In two experiments performed on the same animal on the fifth day, and in another experiment on the seventh day after operation, the critical temperature ranged between  $28^{\circ}$  and  $29.6^{\circ}\text{C}$ ., with no further rise occurring after the fifth day. Very similar results were obtained in the experiments on *animal 15*, no further rise in critical temperature occurring later than the fourth day after operation.

When skin temperature studies were made on animals at intervals greater than

two or three weeks after sectioning the sciatic, or all nerves to the leg, there was found to be a decreasing tendency for the corresponding paw to remain vasodilated and warm. This is in keeping with the observations of Goltz in 1874 and of others (4, 5). Thus, when the animal entered a room which was only moderately cold, the skin of the operated paw often declined in temperature as soon as the normally innervated paws, or its temperature 'floated', gradually shifting up and down at levels somewhat above those of the normally innervated paws. This behavior of the denervated paw is in contrast to the vasodilatation which persisted at moderately low room temperatures, i.e. not cold enough to cool the paw below its 'critical temperature', for weeks or months after (preganglionic) lumbar sympathectomy in the dog we have studied and in the one similarly operated cat followed for a prolonged period.

#### V. *Exclusion of Certain Possible Causes of the Sudden Vasoconstriction*

a) *Adrenal medulla.* That epinephrine may contribute to the vasoconstriction which occurs in blood vessels following severance of the sympathetic nerve fibers, with resulting sensitization to the hormone, has been emphasized by Smithwick (12) and others. Moreover, Elliott (13) and Ascroft (6) have suggested that cold potentiates the action of epinephrine, inasmuch as a given dose of epinephrine will produce a greater drop in skin temperature in a cool extremity than in the same extremity tested while warm. For these reasons it was of primary importance to determine whether sudden vasoconstriction might occur following denervation or removal of the adrenal glands.

In five experiments, both adrenals were denervated previously, or one adrenal was removed and the other denervated (*animals 17, 24, 27*, tables 2 and 3). In three experiments adrenalectomy was performed on the day of the experiment (*animals 9 and 21*, table 2, *animal 23*, table 3). When each of the former group of animals was autopsied, the extent of denervation was found to be almost but not wholly complete, one fine nerve twig running to the adrenal or adrenals from the remaining upper portions of the lumbar sympathetic chains. (The second experiment on *animal 24* was not included in the denervated group because a time sufficient for regeneration had elapsed.) In each of these eight experiments, with the adrenals (almost) totally denervated or removed, there was a fall in the skin temperature of the operated paw which was no less sudden than in the animals with intact adrenals. One of these records, from *cat 21*, Series II, is shown in figure 3, *upper left*. The sudden rise in the temperature of the normal paw following sectioning of the corresponding sciatic nerve provides a check on the adequacy of the blood pressure and the absence of shock.

b) *Possible persistence of nerves to blood vessels.* With the exception of one experiment, in which the skin temperature declined gradually, there was no essential difference between experiments with animals in which the sciatic nerve alone was cut, and experiments with 6 animals, (nos. 18, 20, 21, table 2, and nos. 23, 28, 30, table 3) with total denervation of the leg. The sciatic nerve, femoral nerve and a branch of the obturator nerve were cut and, in addition, in three of these animals the skin and subcutaneous tissues of the leg were cut completely around in the thigh and then sewed up. Portions of the femoral artery and vein were dissected free and soaked

several times with 10 per cent iodine or 95 per cent alcohol. As a final check, in one experiment, (*animal 30*, table 3) four days following section of the nerves to the leg, the leg was completely amputated in mid-thigh, including all tissues and the bone, except for the artery and vein. These vessels were dissected free and were wrapped in cotton soaked with 2 per cent procaine. The bone was wired together and the skin sewed up. As shown in figure 4, sudden vasoconstriction occurred on cooling the box and sudden vasodilatation occurred on subsequently warming the box.

c) *Humoral agents other than epinephrine.* The tendency of a cold environment to cause the body to produce a circulating vasoconstrictor substance was reduced to a minimum in one experiment (the first of 3 on *animal 25*, table 3) by applying heat by means of an electric pad to the body of a cat located in a room at 30°C., whose hind paws, projecting into the cold box, were the only parts of the animal exposed to cold.

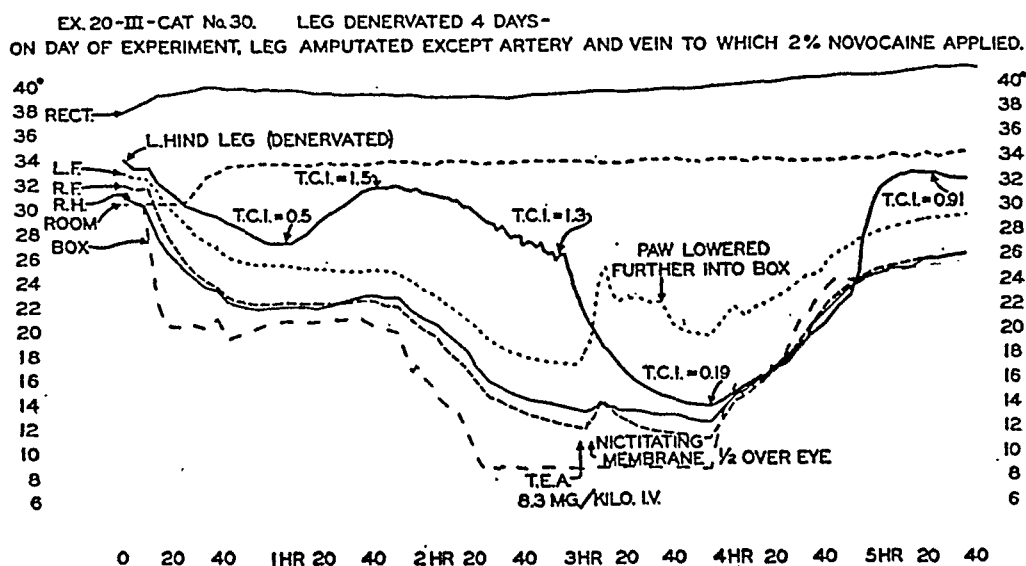


Fig. 4. SUDDEN VASOCONSTRICTION, and later sudden vasodilatation, occurring after exclusion of possible persisting nerves by amputation of leg, with the exception of artery and vein, which were procainized. Both hind paws were alone cooled, but portions of legs above paws were kept warm. Sudden vasoconstriction occurred in previously denervated paw after cooling air in box, and later in the experiment sudden vasodilatation occurred in paw following warming of box.

The chart is shown in figure 5. At the time when the operated paw (sciatic nerve sectioned) showed the characteristic sudden vasoconstriction as a result of cold, the normally innervated paw was warm, indicating absence of reflex vasoconstriction. At the time of the arrow on the chart, the heater pad was turned off, following which the normal paw constricted reflexly as shown. This experiment tends to rule out sympathin as causing the vasoconstriction, inasmuch as generalized sympathetic activity as a result of cold was eliminated by keeping the animal warm. Similarly, the experiment tends to rule out other possible vasoconstrictor substances which might be released as a result of exposure of the animal to cold.

An additional factor tending to rule out sympathin or another vasoconstrictor agent is the sudden vasodilatation occurring in the denervated or sympathectomized limb when the air was warmed, as shown in the latter part of the experiment charted



in figure 4. In the three experiments in which this occurred the normal paw remained reflexly constricted, indicating generalized activity of the sympathetic nervous system in response to cold, yet the sympathin which must have been produced by this activity was incapable of preventing vasodilatation of the operated paw.

Attempts were made to induce the sudden vasoconstriction by injecting various substances or carrying out certain procedures when it was estimated that the skin of the operated paw was close to the 'critical temperature' for sudden vasoconstriction. All such attempts failed to produce the characteristic sudden fall in temperature. The agents and procedures used, with their effects on the skin temperature of the operated paw, were as follows: acetylcholine, 1 to 100,000, sodium bicarbonate, 2 per cent, both I.V., and CO<sub>2</sub> 7.5 and 10.0 per cent, via tracheal cannula (slight rise in skin temperature), ammonium chloride, 2 per cent, I.V., and hyperventilation by means of artificial respiration apparatus, (slight fall in skin temperature).

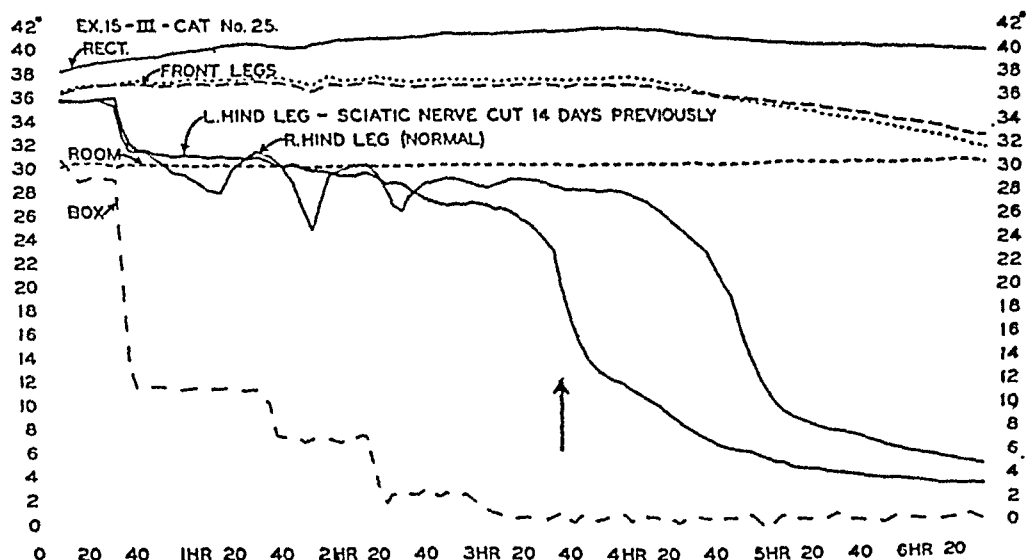


Fig. 5. SUDDEN VASOCONSTRICTION occurring in denervated left hind paw of animal kept warm enough to maintain reflex vasodilatation in normally innervated right hind paw. At the arrow, the heater pad on the animal's body was turned off.

During the course of the investigation, it was found that pentobarbital, when administered in small maintenance doses (approximately 7 mg/kg. intraperitoneally), at a time which happened to coincide with the start of a sudden fall in skin temperature of the operated paw, often actually interrupted the fall, causing the skin temperature to rise again to its previous value for periods of varying duration. In most instances the dose used was insufficient to abolish reflex vasoconstriction. A similar interruption of the sudden fall in skin temperature was produced by means of a moderate dose (7 mg/kg. I.V.) of tetra-ethyl-ammonium bromide ('TEA') administered to an animal with section of all nerves of the brachial plexus (no. 24, table 3). However, in the experiment in which the leg was amputated (fig. 4) there was no interruption of the sudden fall when TEA was injected. These limited data suggest that TEA when it did produce vasodilatation in the operated paw may have done so by preventing impulses from passing down sympathetic nerves persisting in spite of

attempts to eliminate them, rather than via a direct vasodilating action on the blood vessels themselves, especially since TEA has no such vasodilating action when injected intraarterially into normal limbs (14).

#### DISCUSSION

Other factors having been excluded, it is our impression that the sudden vasoconstriction described here may be induced by the combined action of two local processes, as follows: first, as a result of denervation, the smooth muscle of the arteriole may become sensitized to cold, as suggested by Cannon (15). A second factor tending to reduce the blood flow, though not suddenly, may result from a reduced formation of vasodilator metabolites owing to cooling of the tissues, as described by Freeman (18).

As a partial test of the first hypothesis we (16) have studied the effects of cold on the normal and denervated nictitating membrane, a smooth muscle which Bozler (17) places in the same classification ('multi-unit') as the blood vessels, because of certain characteristics in common to both. The nictitating membranes (n.m.) were cooled by means of small glass bulbs which were inserted in place of the eyes of the cats. The effect of cold alone was tested first, by gradually lowering the temperature of water which was pumped through the interior of the bulbs and second, by suddenly lowering the temperature of the water from 37°C. by varying amounts. In two out of four experiments the chronically denervated n.m. showed true sensitization to cold, contracting in response to a smaller drop in temperature than the normal n.m., and showing a greater contraction than the normal with a given drop in temperature for all less-than-maximal contractions. In addition, the contraction of the denervated n.m. in response to cold was always jerky and sudden, producing a stepwise tracing, in contrast to the smooth continuous contraction of the normal n.m.

One would have at least a partial explanation for the sudden vasoconstriction should the chronically denervated blood vessel respond to cooling in the same way as the nictitating membrane. That certain types of blood vessels can become sensitive to local cooling is indicated by the observations of Lewis and Landis (19) in Raynaud's disease, in which arterial vasoconstriction occurred upon exposure to cold even after nerve block or sympathectomy. The fact that sudden vasoconstriction occurred in our experiments after cooling the paw alone, while keeping the leg warm, merely indicates that the response occurred either in the arteries of the paw itself or in the arteriovenous anastomoses of the skin of the paw, but does not distinguish between the two.

#### SUMMARY

A sudden, late fall of skin temperature was observed when denervated or sympathectomized paws of one unanesthetized dog and 16 lightly anesthetized cats were exposed to cold for prolonged periods. A corresponding abrupt rise of skin temperature was observed when the chilled extremities were exposed again to warm air. Analysis of the curves of skin temperature indicated that the vasoconstriction producing this sudden fall in skin temperature was marked enough to reduce blood flow to approximately one-tenth its previous value. The vasoconstriction appeared when

the skin of the operated paws reached a 'critical temperature'. After preganglionic lumbar sympathectomy in the dog, this 'critical temperature' remained constant at approximately  $22^{\circ} \pm 2^{\circ}\text{C.}$ , up to 130 days postoperative. After section of the sciatic nerve in cats, the 'critical temperature' ranged between  $19^{\circ}$  and  $26^{\circ}\text{C.}$  up to 2 days after operation and then rose to reach between 28 and  $31^{\circ}\text{C.}$  by 4 to 21 days postoperative. This effect of prolonged cooling appears to be due chiefly to locally increased sensitivity of the denervated blood vessels to cold because the reaction appeared at the usual 'critical temperature' a) when the adrenal glands were extirpated or inactive, b) when the leg was disconnected from the body except for the procainized artery and vein and c) when only the paws were chilled while the animal's body was kept warm enough to produce maximal vasodilatation in the normally innervated paws.

We wish to thank Dr. Eugene M. Landis for his many helpful suggestions.

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# EFFECTS OF COOLING ON NERVE CONDUCTION IN A HIBERNATOR (GOLDEN HAMSTER) AND NON- HIBERNATOR (ALBINO RAT)

PAUL O. CHATFIELD, ARTHUR F. BATTISTA,<sup>1</sup> CHARLES P. LYMAN<sup>2</sup> AND  
JUANITA P. GARCIA

*From the Departments of Physiology and Anatomy, Harvard University Medical School*

BOSTON, MASSACHUSETTS

LITTLE attention appears to have been paid in recent years to neurophysiological differences between mammals capable of hibernation and those that are not. Horvath (1) pointed out that certain species (ground squirrel, hedgehog, European hamster, and bat) could be cooled artificially until their rectal temperatures approached 0°C. and still revive spontaneously on being returned to a warmer environment. On the other hand cooling to a rectal temperature of 10° to 19°C. is usually fatal to mammals which do not hibernate, for example, rabbits, cats, dogs, guinea pigs, rats and monkeys (2-8), although mice have survived a body temperature of 8.5°C. (9) and newborn rats have recovered from 5°C. (10).

Interest was aroused in the present problem when it was noted that hibernating golden hamsters with body temperatures of 4°C. as measured by rectal and cheek pouch thermocouples still responded to external stimuli. Since it had been shown that nerves of the cat studied *in vitro* cease functioning at about 8°C. (11), it was thought advisable to compare the effects of cooling on conduction by nerve in the hamster and a non-hibernating rodent, the albino rat.

## METHOD

Adult golden hamsters (*Mesocricetus auratus*) and adult albino rats were killed by stunning or decapitation, the tibial nerves removed and placed in Ringer's solution at room temperature. Each of the pair of nerves was then in turn arranged for monophasic recording on silver-silver chloride electrodes in a double-walled moist chamber, the temperature inside of which could be changed in steps from 20°C. to 2°C. by means of circulating brine. The nerve was crushed under the distal recording electrode, but no attempt was made to minimize the positive artefact with KCl or cocaine. A thermometer with bulb close to the nerve measured the temperature inside the chamber. Complete observations were made on 10 nerves from hamsters, 3 of which were from hamsters which had been in hibernation for 32 to 52 days and on 12 nerves from rats.

Preliminary experiments showed that it took about 10 minutes for a nerve to reach equilibrium at any one temperature. Hence the nerves were always left at any given temperature for at least 10 minutes before observations were made.

The nerves were stimulated with supramaximal biphasic shocks from a Grass stimulator led to the nerve in the chamber through a Wagner ground. Nerve action potentials were led into a capacity-coupled amplifier on push-pull and thence to a cathode ray oscilloscope, where the potentials were either photographed or measured directly with a celluloid grid.

Received for publication September 14, 1948.

<sup>1</sup> Porter Fellow of the American Physiological Society.

<sup>2</sup> Research Fellow in Anatomy, Harvard University Medical School.

Since the nerves were short, especially those from the hamsters, we were able to obtain only one elevation of the compound nerve action potential. The variables measured were: height of the action potential, conduction velocity, excitability, the temperature at which the nerve ceased to conduct and, in a few cases, the absolutely and relatively refractory periods. Excitability was measured as the reciprocal of the voltage necessary to just cause the appearance of the nerve action potential. Thus when 'excitability' is used below it refers to the excitability of the fibers with lowest threshold in the two groups of nerves.

All measurements of the above variables made at 20°C. were arbitrarily taken to be 100 per cent and changes in the variables were recorded both in absolute figures and as percentages of the values at 20°C.

Gasser (12) has pointed out that the internal resistance of nerve fibers changes with temperature, which has an effect on the magnitude of the recorded potential. In order to evaluate this source of error, the resistances of tibial nerves from a hamster and rat were measured on electrodes in the chamber with an A.C. bridge at 3000 c/sec. On cooling from 20°C. to 6°C. the nerves showed an average increase in resistance of 61 per cent and the rates of change of resistance were similar. This increase is not great enough to exert significant influence on the results reported below.

TABLE 1. TEMPERATURES AT WHICH THE TIBIAL NERVES OF HAMSTER AND RAT CEASED TO CONDUCT

HAMSTER NERVE	TEMPERATURE, °C.	RAT NERVE	TEMPERATURE, °C.
Exp. H <sub>1</sub>	3.0	Exp. R <sub>1</sub>	7.0
H <sub>2</sub>	2.0	R <sub>2</sub>	9.5
H <sub>3</sub>	3.0	R <sub>3</sub>	7.0
H <sub>4</sub>	3.5	R <sub>4</sub>	12.0
H <sub>5</sub>	3.5	R <sub>5</sub>	8.0
H <sub>6</sub>	2.5	R <sub>6</sub>	11.0
H <sub>7</sub>	6.0	R <sub>7</sub>	10.0
H <sub>8</sub>	3.5	R <sub>8</sub>	9.0
H <sub>9</sub>	3.5	R <sub>9</sub>	7.5
H <sub>10</sub>	3.5	R <sub>10</sub>	10.0
		R <sub>11</sub>	8.5
		R <sub>12</sub>	8.5
Average.....	3.4		9.0

## RESULTS

*Minimal temperatures permitting conduction in nerves of hamster and rat.* The most striking difference between the tibial nerves of the two species studied is that nerves from hamsters as opposed to nerves from rats will conduct when cooled to lower temperatures. Table 1 shows the temperatures at which nerves in each single experiment ceased to conduct.

As can be seen in table 1, the average critical temperature for functioning of nerves was for the tibial of the hamster 3.4°C. and for the rat 9.0°C. Statistical analysis of the figures of table 1 according to the 't' test of significance shows that *P* is much less than 0.01, hence the observations are statistically valid.

*Effect of cooling on height of action potential, conduction velocity, and excitability.* The differences in these respects between the nerves of hamster and rat can best be appreciated by reference to figures 1 to 3, which show typical results. In figure 1 it can be seen that cooling the tibial nerve of a rat (*exp. R10*) from 20°C. causes a progressive decrease in the height of the action potential and in conduction velocity. In figure 3 the relative changes in these variables for the rat are plotted, together with

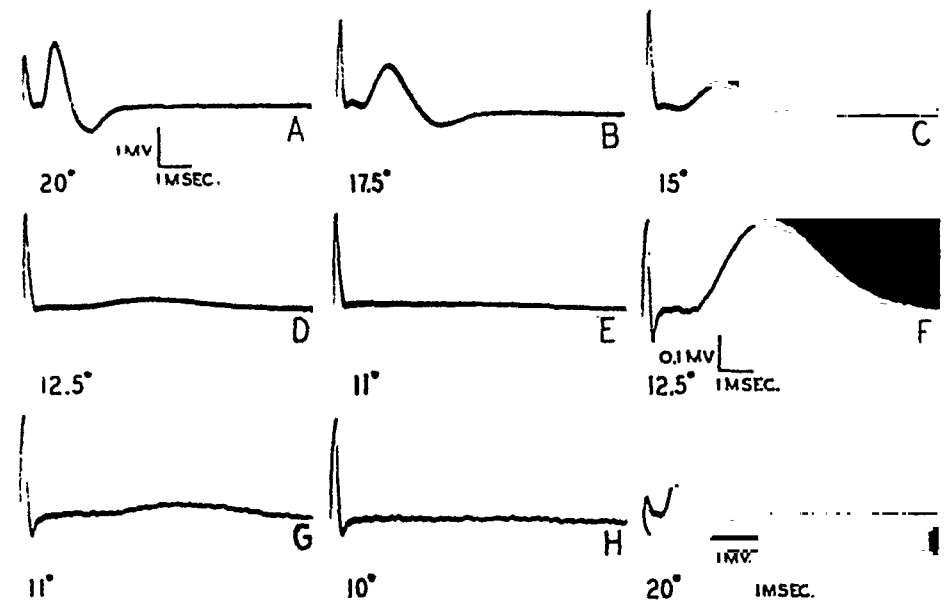


Fig. 1. ACTION POTENTIALS OF TIBIAL NERVE of a rat at various temperatures. *A* through *E* shows gradual decrease in ht. of action potential and conduction velocity on cooling, all at same gain. In *F*, *G* and *H*, with gain increased approximately 10 times, the nerve was cooled from 12.5°C. and the action potential was seen to disappear completely at 10°C. *I*, at original gain, shows the action potential when the nerve had been rewarmed to 20°C. (exp. *R10*).

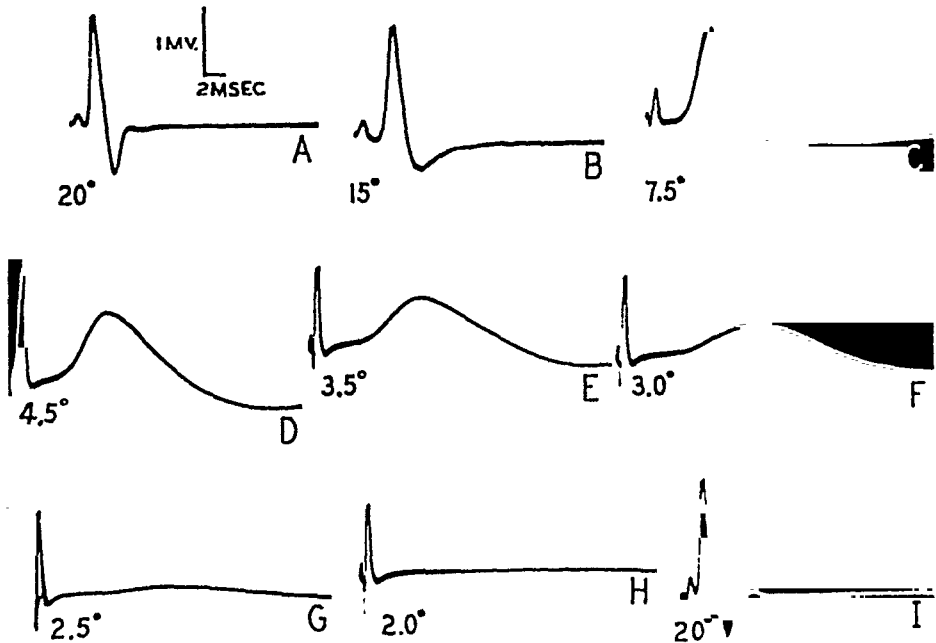


Fig. 2. ACTION POTENTIALS OF TIBIAL NERVE of a hamster at various temperatures. All potentials at same gain. *B* through *H*, gradual decrease in ht. of action potential and conduction velocity on cooling. Note that a small potential was still present at 2.5°C., but disappeared completely at 2.0°C. (confirmed with higher gain). *I* shows the potential when the nerve had been rewarmed to 20°C. (exp. *H2*).

the change in the excitability of the nerve. All three variables are seen to decrease fairly linearly and at approximately the same rate.

Figure 2 shows the action potential of a typical nerve from a hamster (*exp. II2*) which in this case happened to be from a hibernating hamster, although nerves from non-hibernating hamsters behaved similarly. Figure 3 reveals that while again in the hamster there is a fairly linear decrease in conduction velocity and excitability with cooling, the curve is shifted markedly to the left from that of the rat and there is a dissociation in that the action potential actually increases in height transiently as cooling progresses.

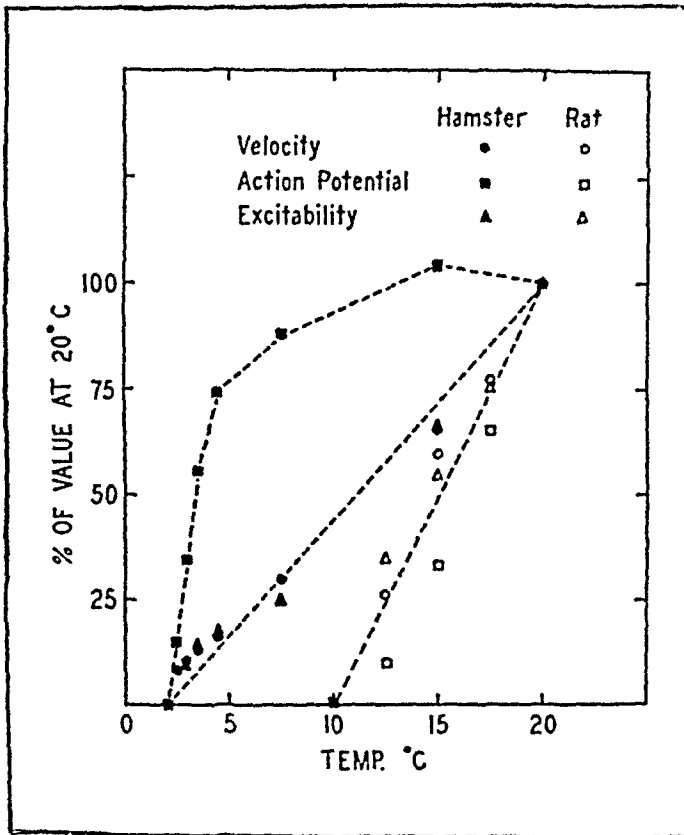


Fig. 3. PLOT OF CONDUCTION VELOCITIES, action potentials, and excitabilities of the nerves of Figs. 1 and 2 at various temperatures, showing the fairly linear decrease in these 3 variables on cooling for the rat, the shifted curve of decrease in conduction velocity and excitability for the hamster and the transient increase in ht. of action potential found in the hamster.

Figures 1 and 2 illustrate one other difference between nerves of hamster and rat, namely, that when the nerves were rewarmed to 20°C. after they had been cooled till conduction ceased, nerves from rats in only 2 out of 12 experiments showed the original height of the action potential, while the nerves of the hamsters in all but one instance showed their original potential or even exceeded it.

*Effect of cooling on absolutely and relatively refractory periods.* These have not been analyzed with precision, but we can state that, as might be expected, cooling increased the duration of these refractory periods. Furthermore, nerves from hamsters required a greater amount of cooling than nerves from rats to bring about the same relative changes in refractory periods.

*Fatigue in cooled nerve of the hamster.* We have noticed in nerves of hamsters that, at temperatures of 5°C., repetitive stimulation at rates as low as 1/sec. resulted

in rapid fatigue as evidenced by a progressive decrease in the size of the action potential.

*Absolute conduction velocities in nerves from hamsters and rats.* Conduction velocities in nerves of hamsters at 20°C. averaged 30.5 m/sec., while nerves from rats at the same temperature conducted at an average rate of 37.8 m/sec. Statistical analysis showed no significant difference between the two groups. Measurement of conduction velocity in such short lengths of nerve is difficult to do accurately, but the highest figure obtained for the rat (69.2 m/sec.) is of the order of magnitude one would expect from consideration of the known maximal fiber diameter in the tibial nerve of this species (13).

*Similarity of function in nerves from hibernating and non-hibernating hamsters.* When it was discovered that nerves from hibernating hamsters conducted at lower temperatures than those from rats, the question arose as to whether the resistance to cold was a property which the nerves acquired as the hamster went into hibernation or whether this property was intrinsic to the species. Subsequent studies revealed no differences in thermal sensitivity between the nerves of non-hibernating and hibernating hamsters. Thus the property of being able to conduct at low temperature is a true species difference and not a change in the nerve which occurs when the hamster is exposed to cold.

#### DISCUSSION

The observations reported here would appear to confirm the dictum of Horvath (1) that "artificial cooling has clearly shown that hibernators react to cooling completely differently than non-hibernators." Indeed Tait (14) stated that a phrenic nerve-diaphragm preparation, as well as the excised heart, from hibernating animals (woodchuck, hedgehog) showed activity at much lower temperatures than would be expected were the preparations from non-hibernating animals.

The fact that the action potential and conduction velocity of mammalian nerve decrease with cooling has been recorded by Gasser (12). If this decrease is assumed to be linear, an extrapolation made of Gasser's figures for the phrenic nerve of the dog shows that this preparation should cease to conduct at about 11°C., which is comparable to our results with the tibial nerve of the rat.

When nerves from hamsters are cooled, conduction velocity and excitability seem to decrease at the same rate, while in the early stages of cooling the height of the action potential is well maintained or may even increase. The literature on the effects of cooling on the height of the action potential is controversial. For example, Gasser (12, 15) described a decline in height on cooling in mammalian and frog nerves, whereas Schoepfle and Erlanger (16) found that cold increased the height of the action potential in frog single-fiber preparations and Lundberg (17) found that cooling increased the height in mammalian C but not mammalian A fibers. At any rate, the behavior of nerve from the hamster on cooling shows a dissociation between height of the action potential and conduction velocity which, if examined in the light of current theories of nerve conduction, indicates that the usual proportionality between these two variables may be more fortuitous than fundamental.

The hamster shows a resistance and adaptation to cold which the rat does not



possess. The mechanism of the differences in response to cooling displayed by nerves from the two species remains obscure. Lundberg (17) has recently shown that C fibers are more resistant to cooling than A fibers. However, it is doubtful that the physiological differences between nerves from hamsters and rats could be based merely on differences in fiber size, since at low temperatures the nerve of the hamster is more excitable and has a larger action potential than one would expect if the still active fibers were only the smallest ones.

Studies on the metabolism of hibernating hamsters in a cold room (4°C.) with a body temperature of 4°C. have shown (18) that, if the room temperature is dropped another 2° to 6°C., the animal's temperature will again begin to decline. Under these circumstances one of three things may happen—the animal's temperature may drop until it dies, the hibernator may 'wake up'<sup>3</sup> or it may remain in hibernation but increase its oxygen consumption so that its body temperature is maintained at 2.5°C.—that is, above the new temperature of the cold room. It is significant that this figure for maintained body temperature is just above the lowest critical temperature for nerve functioning which we have found (2°C. in a hibernator). Hence the hamster shows remarkable adaptation to low temperatures both by a reflex metabolic adjustment for survival and by a tolerance of its nerves for cold not shown by the non-hibernator.

#### SUMMARY

The golden hamster (*Mesocricetus auratus*) hibernates when exposed to cold whereas the rat does not. The behavior of tibial nerves from these two species was studied during cooling to determine whether the effects on the height of the action potential, conduction velocity, excitability and refractory periods would demonstrate species differences in the resistance of nerve to cold.

Nerves from hamsters did not cease functioning until an average temperature of 3.4°C. was reached, while nerves from rats ceased functioning at an average temperature of 9°C. When nerves from rats were cooled, the action potential, conduction velocity and excitability decreased linearly with temperature. When nerves from hamsters were cooled similarly these variables decreased at a slower rate. The action potentials increased in amplitude in the early stages of cooling and then declined.

Cooling increased the duration of the absolutely and relatively refractory periods of nerves from both animals, although relatively less so in the hamster than rat. Tibial nerves of hamsters, though capable of functioning at low temperatures, fatigued rapidly at such temperatures. The critical temperature at which peripheral nerve of the hamster ceases to function is at a level just below that at which hibernating hamsters have been found to maintain their body temperatures by metabolic means when exposed to extreme cold.

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<sup>3</sup> We use the words 'wake up' advisedly. As Horvath (1) said, "Fassen wir alle unsere Kenntnisse über den Winterschlaf zusammen und ziehen besonders den sommerlichen Winterschlaf und unsere volle Unkenntnis des gewöhnlichen Schlafes in Betracht, so gelangen wir immer mehr und mehr zur Einsicht, mit wie viel Recht man (jetzt noch) sagen kann; 'Der Winterschlaf ist erstens kein Schlaf, und zweitens hat er gar nichts mit dem Winter zu thun'".

The results reported are regarded as evidence of an intrinsic adaptation to cold possessed by a species capable of hibernation.

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# HISTAMINE AS THE POSSIBLE CHEMICAL MEDIATOR FOR CUTANEOUS PAIN

SOL ROY ROSENTHAL AND RALPH R. SONNENSCHN

*From the Departments of Preventive Medicine and Public Health, and Clinical Science, University of Illinois College of Medicine*

CHICAGO, ILLINOIS

PREVIOUS studies (1-5) have indicated that histamine, or a histamine-like substance, may be a peripheral mediator of cutaneous pain. By use of sensitive biological methods, it was demonstrated that irritation of the skin or cornea by mechanical, electrical or chemical stimuli, below the threshold for injury, is associated with the liberation of histamine or a histamine-like substance; the quantity liberated varies directly with the intensity of the stimulus. In addition, the perfusion of a solution of histamine onto the denuded skin or its intracutaneous injection is associated with painful sensations.

As a corollary, it was shown that certain histamine antagonists (phenol ethers), in sufficient subcutaneous dosage, produce a generalized peripheral anesthesia in the dog, monkey and human being; on intracutaneous injection, they produce local anesthesia (6). It has since been reported that other anti-histamine drugs, of widely varying structure, likewise act as local anesthetics (7).

A recent study by V. Euler (8) has shown the presence of a relatively high concentration of histamine-like substance in mammalian sensory nerve: 25 to 40  $\mu\text{g}/\text{gm}$ . of nerve. By comparison, only 0.01  $\mu\text{g}$ . of acetylcholine and 1  $\mu\text{g}$ . of sympathin (dS—noradrenaline) per gram were found. Here, again, a rôle of histamine in mediation of pain is suggested.

The present report concerns itself with the determination of the minimum concentration of histamine necessary to produce sensations on intradermal injection. As a control, the actions of acetylcholine, potassium chloride and adenosine, substances which might conceivably play a rôle in pain mediation, were also tested, alone and in combination with histamine.

## METHOD

Twenty-seven adult (male and female) subjects were tested: Caucasian, Negro and Mongoloid racial types were represented in the series. The subjects were members of the faculty of the University of Illinois and, to a lesser extent, of the student body of the College of Medicine.

All solutions used for injection were made up fresh each day, in chemically clean and sterile glassware. The diluent for all test substances was a sterile (pyrogen-free or not) solution of 0.85 per cent NaCl in distilled water, always taken from the flask containing the saline used for the control (blank) injections. Histamine, as the dihydrochloride, and acetylcholine, as the chloride, were

Received for publication October 4, 1948.

diluted directly from sterile ampules. Potassium chloride and adenosine solutions were autoclaved at 15 pounds pressure for 15 minutes.

At the start of each experiment, the subject was put at rest in a comfortable reclining chair and one arm, the volar surface cleansed with soap, water and alcohol, was held in the lap of the experimenter. The subject was not told of the nature of the experiment, other than that there would be a series of intradermal injections, and was not allowed to look at the arm throughout the experiment. He was instructed to state immediately the occurrence of any sensation following the particular injection; this was noted and timed with a stopwatch.

For the injection, 27-gauge needles and 2-cc. syringes were used; a different set was employed for each concentration of the respective solutions. The needle was gently inserted, as superficially as possible, into the skin of the volar surface of the forearm. No injection was made until the pain from this prick had subsided. About 0.01 ml. was injected, producing an initial wheal of 2 to 3 mm. The injections were randomized with saline controls interspersed; there were as many saline placebo injections as of any given solution used. The results of each injection were timed for a minimum of three minutes.

RESULTS

The accompanying table summarizes the essential results. It will be noted that definite painful sensations were reported with histamine at concentrations of  $10^{-15}$  and  $10^{-18}$ ; the results were significant within the 5 per cent limit of confidence ( $P$  value 0.05). In 11 of the 44 trials at these two concentrations, a double sensation occurred, e.g. prickling became a dull pain or vice versa. On the contrary, when saline alone was used, no more than one sensation was experienced, if any, and this was of much shorter duration than that following histamine. This is illustrated by the following protocol of any experiment on a white male subject, using  $10^{-15}$  histamine:

<i>Secs. after injection</i>	<i>Sensation</i>	<i>Secs. after injection</i>	<i>Sensation</i>
0	Nothing	100	Pain decreasing
30	Low intensity pain	120	Very slight pain still present
60	Increasing pain, same quality as above	150	No pain, but 'awareness' at site of injection
83	Pain still present	170	All sensation gone
90	Pain at maximum		

With the same individual, one response to saline was entirely negative and the other two were as follows:

<i>Secs. after injection</i>	<i>Sensation</i>	<i>Secs. after injection</i>	<i>Sensation</i>
0	Nothing	60	Nothing
15	Nothing	90	Nothing
30	Nothing	120	Nothing
45	Slight pain of very low intensity		

Generally, at the higher concentrations of histamine,  $10^{-8}$  and greater, three types of sensations were reported with each injection.

Itching, usually following an initial pain, occurred with concentrations of  $10^{-8}$  and greater, but not at the lower concentrations. Secondary wheals and flares

TABLE 1. RESPONSE TO HISTAMINE INJECTED INTRADERMALLY (27 NORMAL ADULTS)

CONCENTRATION <sup>1</sup>	$\times 10^{-5}$ (2.5 to 1.25)		$\times 10^{-4}$ (10 to 3.1)		$10^{-3}$ to $10^{-2}$		$10^{-2}$ to $10^{-1}$		$10^{-1}$ to $10^{-10}$		$10^{-10}$		$10^{-11}$		$10^{-12}$		Saline	
Type of sensation	Obs. <sup>2</sup>	Pos. <sup>3</sup>	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.	Obs.	Pos.
	10		10		54		11		26		24		20		60			
<i>Prickling, stinging or tingling.</i>																		
As 1st sens. <sup>5</sup> .....		1		2		15		3		3		5		6		7		
as 2d sens. ....		0		1		3		2		0		2		0		0		
as 3d sens. ....		0		0		2		0		0		0		0		0		
		—		—		—		—		—		—		—		—		
Total. ....		1		3		20		5		3		7		6		7		
<i>Burning</i>																		
As 1st sens. ....		0		1		9		0		3		3		3		4		
as 2d sens. ....		6		3		1		0		0		0		0		0		
as 3d sens. ....		1		0		0		0		0		0		0		0		
		—		—		—		—		—		—		—		—		
Total. ....		7		4		10		0		3		3		3		4		
<i>Itching</i>																		
As 1st sens. ....		0		1		4		1		0		0		0		3		
as 2d sens. ....		4		3		9		0		0		0		0		0		
as 3d sens. ....		4		2		2		0		0		0		0		0		
		—		—		—		—		—		—		—		—		
Total. ....		8		6		15		1		0		0		0		3		
<i>Pain (dull)</i>																		
As 1st sens. ....		2		1		11		1		3		7		2		7		
as 2d sens. ....		0		1		7		0		2		1		2		0		
as 3d sens. ....		0		9		0		0		0		0		0		0		
		—		—		—		—		—		—		—		—		
Total. ....		2		11		18		1		5		8		4		7		
<i>Pain (sharp)</i>																		
As 1st sens. ....		7		5		9		2		2		3		2		0		
as 2d sens. ....		0		1		6		0		1		5		1		0		
as 3d sens. ....		0		0		0		0		0		0		0		0		
		—		—		—		—		—		—		—		—		
Total. ....		7		6		15		2		3		8		3		0		
Grand total...	10	10 <sup>4</sup>	10	10	54	48	11	7	26	11	24	18	20	13	60	21		
Percentage positive. 100%			100%		89%		63%		42%		75%		65%		35%			
Value of $p^6$ .....	<0.01		<0.01		<0.01		<0.05		<0.05		<0.01		.05-.01					

<sup>1</sup> Concentrations are calculated for histamine base.<sup>2</sup> Obs. = Number of observations (individual injections); <sup>3</sup>Pos. = Number of positive responses.<sup>4</sup> Grand total of positives is sum of positive injections without regard to number of sensations produced.<sup>5</sup> 1st, 2d and 3d sensations refer to order of appearance of respective sensations as reported by subject after each injection.<sup>6</sup>  $p$  is calculated from chi-square of fourfold table, with grand total figures for each concentration of histamine compared with those for saline.

(triple response) were also absent with concentrations less than  $10^{-8}$ ; when present, they were not always accompanied by itching (9). No correlation in sensitivity to histamine was found with either sex or race.

Potassium chloride alone at 1:100 caused a sharp stinging, while 1:1000 dilution gave no sensation. Acetylcholine at 1:500 produced a definite burning sensation but little to no sensation at 1:1000 dilution; when combined with histamine, at a final dilution of 1:1000, it did not augment the pain-producing action of histamine. A similar lack of augmentation was found with adenosine (1:2000 final dilution).

#### DISCUSSION

By application of Avogadro's Number, it may be shown that 0.01 ml. of  $10^{-18}$  histamine base contains 54 molecules of the active agent. Errors in the serial dilution may, of course, cause the actual number to vary by 100 to 200 per cent. Even so, since such an extremely small number of molecules is associated with definite painful sensation, the postulate of specificity of histamine (or a histamine-like substance) as an integral part of the peripheral cutaneous pain mechanism is strongly supported. The possibility remains that the physiologic substance is only 'histamine-like', and that the injected histamine simulates this substance. The decision on this point must remain in abeyance until more specific methods are available for characterizing the naturally occurring substance, released on painful stimulation.

Our results on the action of acetylcholine are in variance with those of Emmelin and Feldberg (10), who reported that this substance, injected into the skin by puncture with a sharp needle, at 1:50 and 1:100 caused no sensation, but in combination with histamine caused a burning sensation. The discrepancy in observed responses may be due to the difference in methods of injection.

Our results indicate that itching is not a 'subthreshold' pain, since production of an itch requires a stimulus much stronger than that for a pain. It appears, rather, that the sensation (s) variously described by our subjects as 'prickling', 'tingling', or 'stinging' is most likely the least perceptible manifestation of pain.

#### SUMMARY

Painful sensations may be produced by the injection of histamine, at concentrations as low as  $10^{-18}$ , into the superficial layers of the cutis. These findings are taken to indicate specificity of histamine in production of cutaneous pain and substantiate the postulate that a histamine-like substance acts as a physiologic mediator of pain. Acetylcholine and adenosine apparently do not augment the cutaneous pain-producing action of histamine. The fact that production of itching requires a higher concentration of histamine than that necessary for pain indicates that itching is not a 'subthreshold' pain. The least perceptible manifestations of pain are 'prickling', 'stinging' or 'tingling'.

We acknowledge with thanks the help and suggestions given by Dr. Carl C. Pfeiffer, Head of the Department of Pharmacology, University of Illinois.

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# RELATION OF CEREBRAL ARTERIOVENOUS DIFFERENCE IN OXYGEN CONTENT TO ARTERIAL CARBON DIOXIDE TENSION

E. S. GURDJIAN, J. E. WEBSTER AND W. E. STONE<sup>1</sup>

With the technical assistance of J. KOPALA

*From the Laboratory of Surgical Research, Wayne University College of Medicine, and the  
Department of Neurosurgery, Grace Hospital*

DETROIT, MICHIGAN

LENNOX, Gibbs and co-workers (1-3) have published a series of investigations leading to the theory that the brain is protected from rapid alteration of its acid-base balance by compensatory changes in the cerebral blood flow. This regulatory mechanism is considered to be functional only in the presence of adequate oxygen, the need for oxygen being paramount. The theory is based in large part on studies of cerebral arteriovenous differences as observed in human subjects, the cerebral venous blood being obtained from the internal jugular vein.

This theory was questioned by Schmidt and co-workers (4, 5), who measured the cerebral blood flow and gaseous metabolism in the monkey and concluded that the tone of the cerebral vessels tends to alter in accordance with the oxygen requirement of the brain, which varies with its functional activity. Gibbs, Maxwell and Gibbs (3) pointed out that the values for oxygen content of the blood appeared to be considerably below normal in the experiments of Schmidt and co-workers. Geiger and Magnes (6), using the perfused brain of the cat, obtained results which agreed in part with those of Schmidt and co-workers. They found that the cerebral blood flow could be correlated with oxygen consumption at the lower levels of blood flow, but not at the higher levels. Carbon dioxide showed only a slight regulatory effect on blood flow. In a study of the effects of hyperventilation on human subjects, however, Kety and Schmidt (7) reached conclusions which are in accord with those of Lennox and co-workers.

Some of the interpretations of Lennox, Gibbs and Gibbs and of Kety and Schmidt have been called into question by Ferris and his co-workers (8), who, while not disputing the regulatory function of carbon dioxide, found that variable amounts of extracranial blood may be included in specimens drawn from the internal jugular vein

In this paper are presented some measurements of cerebral A-V differences in oxygen content obtained on dogs. Blood samples were obtained from the superior sagittal sinus representing the blood draining the cerebral cortex without admixture of significant amounts of extracranial blood. These data have been obtained in the course of studies on metrazol convulsions (9) and on the relations between the blood gases and certain constituents of the cerebral tissue (10). The findings are in agreement with the views of Lennox and co-workers.

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Received for publication October 4, 1948.

<sup>1</sup> Present address: Department of Physiology, University of Wisconsin, Madison, Wis.



## PROCEDURES

Dogs were given 20 mg. morphine sulfate/kg. body weight by subcutaneous injection. A Magill intratracheal tube with a Waters-Guedel inflatable cuff was inserted. Local infiltration with 0.25 per cent procaine hydrochloride was used for exposure of the skull and of the femoral arteries and veins. A trephine opening was made over the superior sagittal sinus and the bone of the calvarium was removed over a wide area, leaving the dura intact. One femoral artery was cannulated for the measurement of blood pressure. At this point the 'normal' blood specimens were drawn simultaneously from the femoral artery and the sagittal sinus, being collected anaerobically over mercury with heparin as the anticoagulant.

Mixtures of oxygen, carbon dioxide and nitrogen were administered from a 5-liter breathing bag connected to a Foregger anesthesia apparatus. During the period of administration of the desired mixture, 12 to 14 minutes after its inception, blood specimens were again taken simultaneously from the artery and the sagittal sinus.

Decreased arterial carbon dioxide tension was attained by hyperventilation with air or oxygen. In this group of experiments the animals were immobilized by intravenous injection of a 0.75 per cent solution of dihydro-beta-erythroidine hydrobromide<sup>2</sup> in physiological saline (1 cc/kg. body weight), the dose being sufficient to induce respiratory paralysis. Artificial respiration was then given by means of a respiratory pump.

*Blood Gases and pH.* The oxygen content and capacity and the carbon dioxide content of the blood were determined by the manometric methods of Van Slyke and Neill (11). Blood pH was determined anaerobically at 38° C. within a few minutes after the specimen was drawn, by means of a glass electrode and a voltmeter similar to that described by Nims (12). The standard buffers used were those of Hastings and Sendroy (13), since it was desired to calculate the carbon dioxide tension from the line charts of Van Slyke and Sendroy (14) based on the same pH scale. Root *et al.* (15) have shown that these charts are applicable to dog blood.

## RESULTS

The cerebral arteriovenous difference in oxygen content was found to be greatly increased when the arterial carbon dioxide tension was decreased by hyperventilation. Likewise a decrease in the A-V difference occurred when the carbon dioxide tension was increased. In the 'normal' animals (breathing air), the A-V difference was found to be inversely related to the arterial carbon dioxide tension. In figure 1 are plotted all the available data except those in which the arterial oxygen tension was below 28 mm. Hg (calculated from the oxygen dissociation curve, with appropriate pH corrections). It is evident that when the oxygen supply is adequate the A-V difference in oxygen content is a function of the arterial carbon dioxide tension. The curve shows the upper and lower limits of the A-V difference, which may be interpreted as indicating the physiological limits of the cerebral vascular mechanism in response to changes in carbon dioxide tension.

<sup>2</sup> Courtesy of Merck and Company, Inc., Rahway, N. J.

It is worthy of note that the paralyzing drug dihydro-beta-erythroidine hydrobromide is without noticeable effect on the responsiveness of the cerebral circulation to variations in carbon dioxide tension. In four animals of this group there was a tendency for the blood pressure to decrease to low levels during hyperventilation, but this was counteracted by the intermittent injection of adrenaline in amounts just sufficient to maintain the blood pressure in the normal range. In two hyperventilated animals the attempts to obtain sufficient blood from the sagittal sinus were unsuccessful, apparently as a result of extreme vasoconstriction and decreased blood flow.

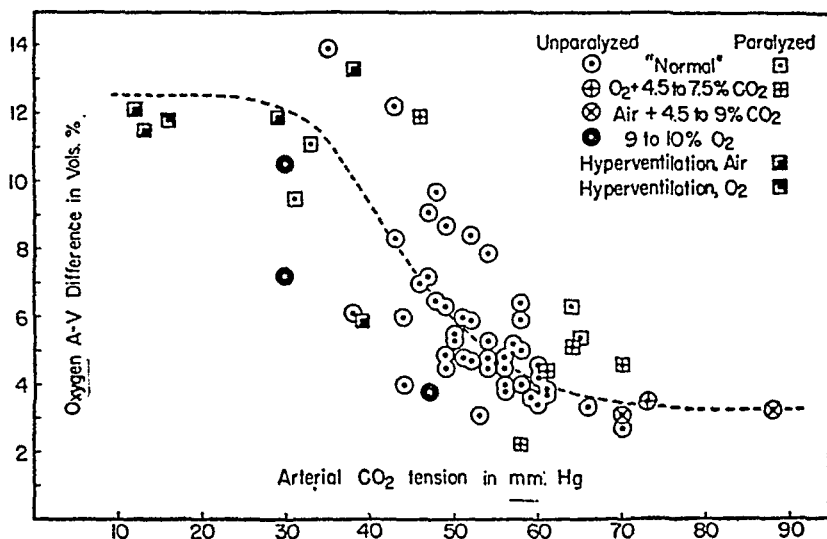


Fig. 1. RELATION OF THE CEREBRAL A-V OXYGEN DIFFERENCE to the arterial carbon dioxide tension.

#### DISCUSSION

Changes in the A-V oxygen difference induced by variations of carbon dioxide tension might be due to changes in either the cerebral blood flow or the cerebral oxidative rate, or to a combination of these factors. However, the available evidence indicates that changes in oxidative metabolism play only a minor rôle at most. Gibbs, Maxwell and Gibbs (3) found in the human being that increasing the carbon dioxide decreased the cerebral oxidations to some extent, but the effects of decreasing the carbon dioxide level were ambiguous. Schmidt (16) found that excess carbon dioxide decreased the apparent metabolism of the perfused dog's head in three of four experiments, but a similar effect induced by adrenaline was attributed to constriction of vessels in extracranial tissues. Kety and Schmidt (7) found in human subjects that active (voluntary) hyperventilation increased the cerebral oxygen uptake by 15 per cent, while passive hyperventilation caused no change. On the other hand, Geiger and Magnes (6), using the perfused cat's brain, found that increased carbon dioxide tension increased the oxygen consumption when the flow rate was originally low, the effect being secondary to an increase in blood flow. These findings appear to justify

the interpretation that the observed changes in the cerebral A-V oxygen difference resulting from alterations of carbon dioxide tension are due in large part to changes in blood flow through the cortex.

#### SUMMARY

Simultaneously drawn specimens of arterial and cerebral venous blood were obtained from morphinized dogs. Since the venous blood was obtained from the superior sagittal sinus it represents blood draining the cortex without admixture of significant amounts of extracranial blood. The specimens were analyzed and values calculated for carbon dioxide and oxygen tensions and for A-V difference in oxygen content. The effects on these variables of changes in the respiration or in the composition of the respired air were observed.

In the presence of adequate oxygen the cerebral A-V oxygen difference is a function of the arterial carbon dioxide tension. The A-V difference is high when the carbon dioxide tension is low, and vice versa. The data support the view of Lennox, Gibbs and co-workers that the brain is protected from rapid alteration of its acid-base balance by compensating changes in the cerebral blood flow.

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# ACCELERATORY EFFECTS ON RENAL FUNCTION<sup>1</sup>

H. SILVETTE AND S. W. BRITTON

With the technical assistance of C. E. ARMENTROUT

*From the Physiological Laboratory, University of Virginia Medical School*

CHARLOTTESVILLE, VIRGINIA

**P**HYSIOLOGICAL stresses to which the organism is subjected in aviation are frequently referred to low barometric pressures or acceleratory forces which may be involved. Renal changes in animals exposed to simulated high altitudes have been investigated by one of us (1) and other work from this Laboratory has suggested that the kidneys may be affected during or after tests on acceleration. The present experiments were designed to investigate possible renal changes in function and structure resulting from exposure to various positive and negative acceleratory forces.

## METHODS

Adult male white rats of Wistar strain were used throughout. Urine collection was made in small glass graduated cylinders under individual metabolism cages and the chloride content analyzed by the standard Volhard titration. Residual bladder urine was expressed by manual pressure and massage technique.

The animals were subjected to acceleratory forces on a centrifuge of 10-foot radius (2). A special animal board which held 6 rats in the supine position was used; the strain on the body was partly borne by a soft leather band with two holes to accommodate the forefeet. Front and hind legs were extended and tied. In this position rats could be held without apparent discomfort for considerable periods of time if desired. During runs on the centrifuge the animals were covered by a cloth to protect against cooling by air draughts. Control tests showed that simply holding normal animals tied in the supine position for time periods similar to those of the experimental runs had no influence on fluid or chloride output. All injections were made intraperitoneally on the basis of body weight of the animal, with fluid (0.2% or 0.5% sodium chloride solution) warmed to body temperature.

The animal board was reversible and positive or negative *g* could be applied to the experimental group as a whole. A simultaneous paired control was run in each experimental group, all factors being duplicated except acceleration.

## RESULTS

*Positive g: urine secretion during acceleration.* All animals were given 7.5 cc. of 0.5 per cent NaCl/100-gm. body weight in order to furnish ample fluid for secretion. At the end of two hours the urinary bladders were emptied by gentle pressure massage and the animals then tied down on boards in groups as indicated. One group was centrifuged at 2 *g* for a period of 60 minutes, precaution being taken by suitable ligation (under novocaine) that urine was not voided during the exposure

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Received for publication October 7, 1948.

<sup>1</sup> The experiments described were undertaken as part of a program of research performed under contract with the U. S. Navy, Office of Naval Research and the University of Virginia.

TABLE 1. ACCELERATION AND RENAL SECRETION. POSITIVE (+) G FORCES

Group I<sup>1</sup>

NO. TESTS	EXPERIMENTAL CONDITION	URINE OUTPUT	
		cc. in 60 min.	% of injected fluid
10	Control (no accel.)	1.1	18.2
11	Acceleration	0.2	1.7

Group II<sup>2</sup>

NO. TESTS	EXPERIMENTAL CONDITION	URINE EXCRETION AT END OF		5-HR. CHLORIDE EXCRETION
		3 hrs.	5 hrs.	
6	Control (no accel.)	cc. 0.2	cc. 0.6	mg. 3.1
5	Acceleration	0.6	0.9	3.8

Group III<sup>3</sup>

SERIES	NO. TESTS	EXPERIMENTAL CONDITION	URINE EXCRETION AT END OF				
			2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.
			cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.	cc/100 gm. b.wt.
A	12	Control (no accel.)	1.6	1.9	2.6	3.0	3.5
	12	Acceleration (repeated)	1.9	2.4	2.9	3.1	3.5
B	21	Control (no accel.)	1.0	1.6	2.1	2.8	3.5
	22	Acceleration (contin.)	1.4	2.3	3.0	3.6	4.3

Group IV<sup>4</sup>

SERIES	NO. TESTS	EXPERIMENTAL CONDITION	3-HOUR URINE OUTPUT	CHLORIDES	
				Concentration	Total 3-hr. excretion
			cc/100 gm. b.wt.	mg/cc.	mg.
A	12	Control (no accel.)	2.3	0.59	1.4
	12	Acceleration (repeated)	3.0	0.55	1.7
B	28	Control (no accel.)	2.1	1.16	2.5
	28	Acceleration (contin.)	2.8	0.89	2.5

<sup>1</sup> Animals given 7.5 cc. 0.5% NaCl/100 gm. b. wt.; 2 hr. later, experimental rats exposed to 2 g for 60 min.

<sup>2</sup> *Ad lib.* fluid intake; exposure, 5 g for 15 sec., 30 times at 1-min. intervals.

<sup>3</sup> Accel. (A), 5 g for 15 sec., 15 runs at 1 min. intervals, or (B) 2 g for 15 min.; rats injected with 5 cc. 0.5% NaCl/100 gm. b. wt.

<sup>4</sup> Accel., 5 g for 15 sec., (A) repeated 25 times at 1 min. intervals, or (B) 2 g for 15 min.; rats given 5 cc. 0.2% NaCl/100-gm. b. wt.

period; the others were similarly tied down only to serve as non-accelerated controls. At the end of the 60-minute experimental period the rats were killed, the urinary bladders clamped off and removed and the contained fluid measured. From the

results shown in table 1 I and figure 1, it will be observed that during exposure to  $g$  forces urinary secretion is virtually stopped.

*Urine secretion after exposure to  $g$ .* Rats which had been allowed free access to food and water were a) centrifuged for 15 minutes at 2  $g$ , or b) given 30 runs at 5  $g$  for 15 seconds at 1-minute intervals; each group was then placed in metabolism cages. There was no significant difference between the urine output of group a rats and their controls, but in the b series there was a greater post-acceleratory renal output in the experimental animals at the end of three hours. During the succeeding two hours, accelerated and control animals both excreted about the same amount of urine. No

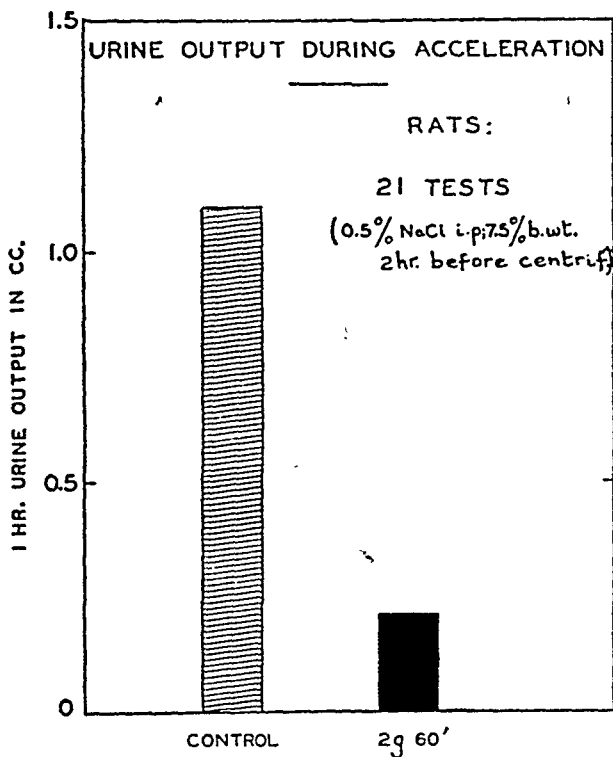


Fig. 1

significant difference was noted, also, between the five-hour chloride output of the two groups (table 1, II).

Though the percentage increase in urine output of accelerated animals (5  $g$ , 15 sec., 30 runs) over the controls was high, the actual quantity of urine excreted was of a small order on an *ad libitum* water intake. A group of animals was therefore rendered diuretic by the injection of sodium chloride solutions immediately after exposure to  $g$  forces and before placing them in metabolism cages.

Groups of animals were exposed 15 times to 5  $g$  for 15 seconds at one-minute intervals, then injected intraperitoneally with 5 cc. of 0.5 per cent NaCl/100 gm. body weight and placed in metabolism cages. Non-accelerated controls were similarly injected and the urine output was followed during the succeeding six hours. Other animals were subjected to 15 minutes' acceleration at 2  $g$  (fig. 2). Results are given in table 1, III, A and B. Urinary secretion of the repeatedly accelerated animals was considerably higher than that of the controls over several hours in the A

series, while in the *B group* post-acceleratory output was maintained at about 40 per cent higher over the six-hour observation period.

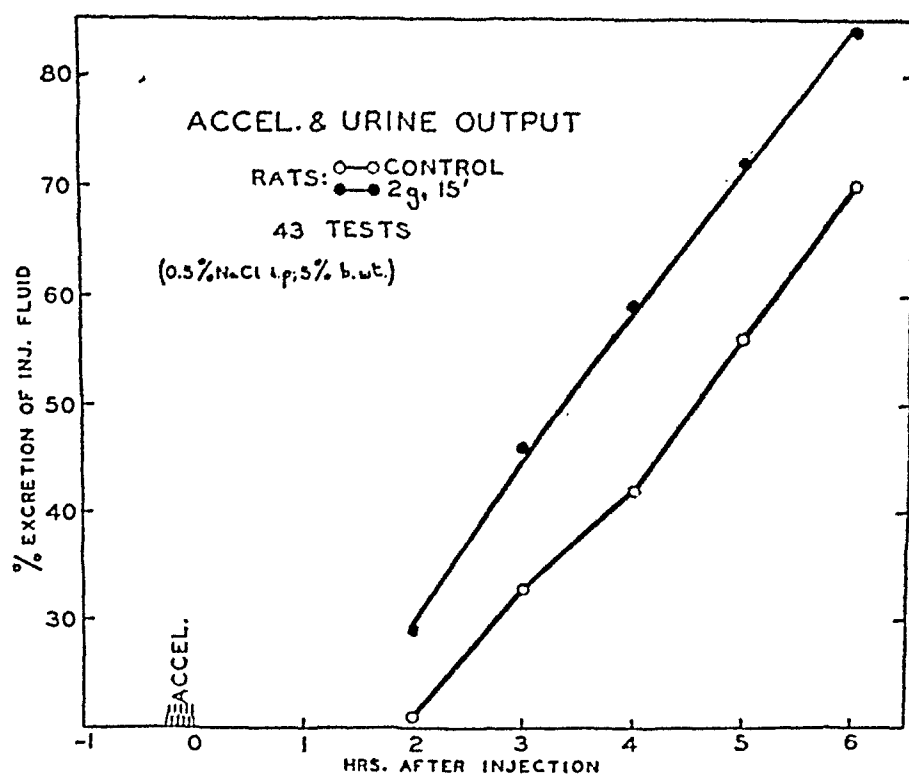


Fig. 2

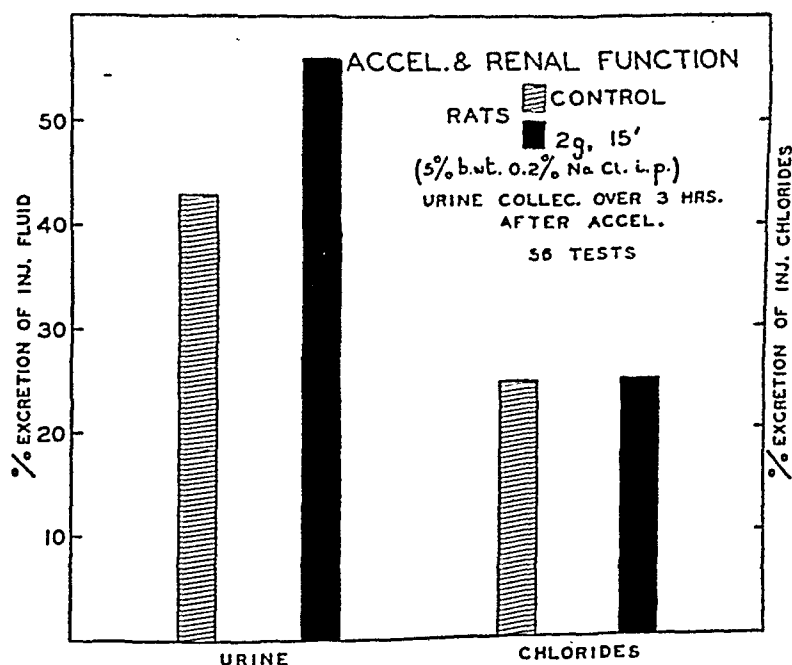


Fig. 3

Animals subjected to either repeated acceleration (5 g, 15 sec. for 25 runs at 1-min. intervals) or a continuous exposure period (2 g for 15 min.) were injected with

5 cc. of 0.2 per cent NaCl/100 gm. body weight, placed immediately in metabolism cages and the urine collected and analyzed for chlorides at the end of three hours. Urine output was increased over the similarly injected controls and chloride concentration diminished in both series (table 1, IV and fig. 3).

*Effect of long periods of acceleratory exposure.* Several groups of animals were exposed to various positive and negative *g* forces over periods of 8 to 24 days (daily

TABLE 2. RENAL AND OTHER CHANGES FOLLOWING ACCELERATORY EXPOSURE

SERIES	NO. RATS	EXPERIMENTAL CONDITION	B.WT. AT BEGINNING OF EXPOSURE	B.WT. AT END OF EXPOSURE	GAIN IN B.WT.	WT. BOTH KIDNEYS	KIDNEY WT/B.WT.
			gm.	gm.	%	gm.	
A	5	Control (no accel.)	190	249	31	2.02	0.81
	6	Acceleration (+2 g for 15 min.; 17 days)	178	205	16	1.72	0.84
B	6	Control (no accel.)	192	212	10.4	1.63	0.77
	6	Acceleration (-4 g for 15 min.; 17 days)	187	196	4.8	1.49	0.76
C	3	Acceleration (+6 g, 15 sec. 3 runs daily, 8 days)	229	237	3.5	1.70	0.72
	3	Acceleration (+6 g, 15 sec. 3 runs daily, 24 days)	227	254	11.8	1.93	0.76

TABLE 3. ACCELERATION AND KIDNEY FUNCTION: EFFECT OF PITRESSIN<sup>1</sup>

SERIES	PITRESSIN	CONTROLS (NO ACCELERATION)		ACCELERATION (2 G, 15 MIN.)	
		3-hour urine output	Urinary chlorides	3-hour urine output	Urinary chlorides
	meg.	cc/100 gm. b.w.	mg/cc.	cc/100 gm. b.w.	mg/cc.
1	0	2.1	0.33	2.7	0.48
2	4	1.3	0.71	2.1	0.63
3	8	1.1	2.54	1.8	1.52
4	16	0.4	2.62	1.9	1.64
5	32	0		0.8	2.40
6	63	0.4	8.80	0.4	7.11
7	125	0.6	11.6	0.7	10.6
8	250	1.4	8.31	1.5	9.12
9	500	1.8	8.67	1.7	8.00
10	1000			1.3	9.32

<sup>1</sup> All animals given 5 cc. 0.2% NaCl/100-gm. b. wt. plus amount of pitressin noted; 6 or more tests in each series.

tests, except week-ends). At the end of these periods the animals were killed and the kidneys removed, weighed and sectioned for histological analysis. During the experimental period, the exposed animals gained less weight than the controls, although the ratio of kidney to body weight was insignificantly higher (table 2, A, B, C). Tests for urinary albumen at the end of the test periods were almost invariably positive in these cases. Histological examination revealed that the renal glomeruli



of the accelerated animals were ischemic and the proximal convoluted tubules showed cloudy swelling and contained casts. The loop of Henle was apparently unaffected.

*Effect of post-pituitary extract on acceleratory polyuria.* Groups of animals were subjected to acceleratory forces of 2 g for 15 minutes, injected with 5 cc. of 0.2 per cent NaCl solution, containing various amounts of pitressin, and then placed in metabolism cages for a period of three hours. At the end of this period the urine was measured and its chloride concentration determined (table 3).

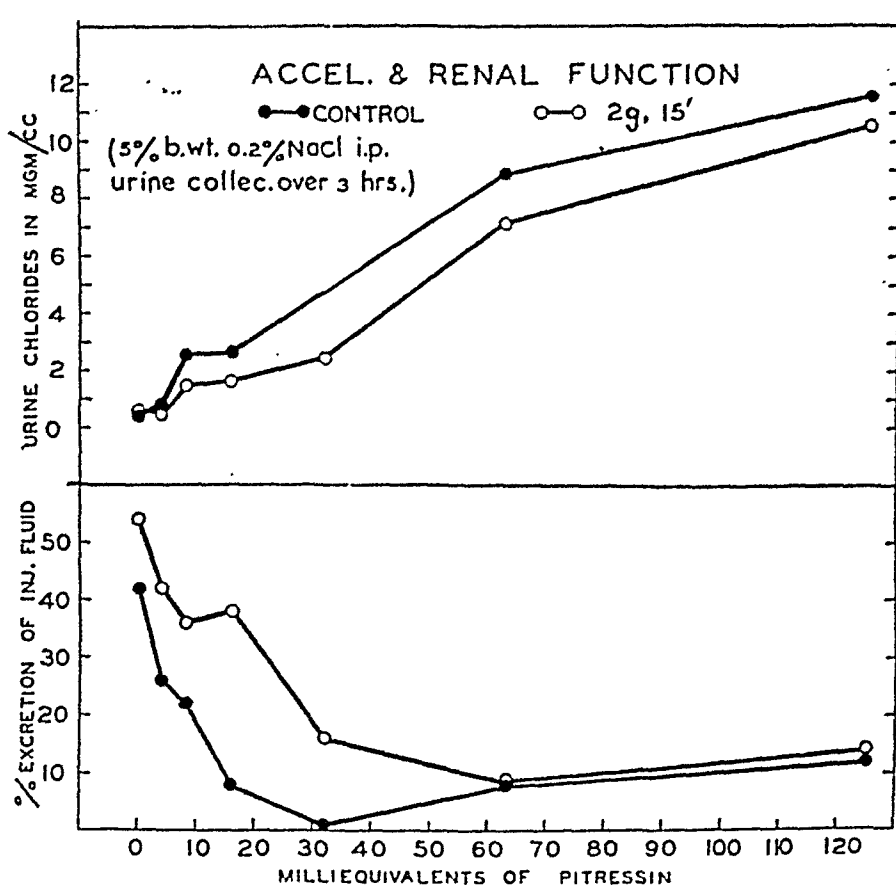


Fig. 4

Curves showing the effect of post-pituitary extract on the urine and chloride output of non-accelerated rats followed the usual pattern previously observed and described by one of us (3). With decreasing doses of pitressin, the urine output gradually rose while its chloride concentration fell. The most abrupt fall in chloride concentration was observed in earlier work on using a pitressin concentration of approximately 4 mU/100-gm. rat. In the present tests a similar sharp fall in chloride concentration of the urine taken from accelerated rats paralleled closely (though it was somewhat below) that of control animals (fig. 4).

As the dose of pitressin was decreased (for convenience the dose was halved in each successive experiment), the curves of urine secretion of accelerated and control animals began to diverge on using approximately 60 mU. (fig. 4). At this point acceleratory polyuria 'broke through' post-pituitary inhibition of urine flow. It may

be noted that with this concentration of pitressin, urinary chlorides were still unaffected. Thus in both non-accelerated and accelerated animals the chloride-concentrating power of the hormone was better maintained than its water-concentrating power.

*Negative g: urine secretion during acceleration.* In these experiments the factors of technique time, fluid injection and metabolic period were the same as those previously described under positive g, but the animals were exposed to  $-4$  g instead of  $+2$  g for 60 minutes. The results were similar, i.e. during exposure to negative g (as well as positive g) urine secretion was almost completely suppressed (table 4, I).

TABLE 4. ACCELERATION AND RENAL SECRETION. NEGATIVE (—) G FORCES

Group I<sup>1</sup>

NO. TESTS	EXPERIMENTAL CONDITION	URINE OUTPUT	
		cc.in 60 min.	% of injected fluid
4	Control (no accel.)	1.5	10.0
6	Acceleration	0.2	1.4

Group II<sup>2</sup>

NO. TESTS	EXPERIMENTAL CONDITION	3-HOUR URINE OUTPUT	CHLORIDES	
			Concentration	Total 3-hr. excretion
		cc/100 gm. b.wt.	mg/cc.	mg.
18	Control (no accel.)	2.2	1.39	3.1
18	Acceleration	3.1	0.52	1.6

<sup>1</sup> Animals given 7.5 cc. 0.5% NaCl/100-gm. b. wt.; 2 hours later, experimental rats exposed to  $-4$  g for 60 min.

<sup>2</sup> Accel.,  $-4$  g, 15 min.; animals given 5 cc. 0.2% NaCl/100-gm. b. wt. i.p.

*Urine secretion following negative acceleration.* Rats which were exposed to  $-4$  g for 15 minutes, then injected with 5 cc. of 0.2 per cent NaCl/100-gm. body weight and placed in metabolism cages for three hours, excreted more urine and less chloride than their controls (table 4, II). The increase in polyuria over the controls was 36 per cent after exposure to negative g, 33 per cent after positive g—an insignificant difference. Urinary chloride concentration was reduced 62 per cent from the control level following  $-4$  g, only 23 per cent after  $-2$  g—a difference which appears to be significant.

## DISCUSSION

Although the acceleratory exposures given in the present experiments were usually more severe than those experienced by man, they could not be considered critical for rats. No animal died from the acceleration and the general condition of animals following the tests was excellent, even when exposures were made daily over periods of a few weeks. Considered in relation to earlier results on the effects of

high altitude exposure on kidney function and renal pathology, the results of acceleratory exposures appear very interesting and significant.

Rats exposed to low barometric pressure (25,000 ft. equiv. alt.) have been observed to suffer much greater polyuria than those subjected to high acceleratory forces; also, the pathological changes in the kidneys following repeated exposure to each condition were strikingly different. The kidneys of 'high-altitude rats' were greatly hypertrophied, the glomeruli were congested and slight tubular pathology was observed. In the case of the *g*-exposed rats, however, there was little or no gross renal hypertrophy, the glomeruli were characterized by almost complete absence of blood and the tubules by cloudy swelling, while the loop of Henle presented a normal appearance.

It appears difficult to explain or correlate the pathological changes observed after repeated exposure to acceleration with the changes in renal function as reported herein. However, the functional changes described would seem to be referable to both glomerular and tubular impairment consequent upon disturbances in renal circulation produced by high acceleratory forces, with resultant anoxia of the intimate renal tissues.

Changes in kidney secretion observed following centrifugation, it should be considered, may be due either to *a*) stimulation and enhanced secretion of the cortico-adrenal tissues or *b*) to central inhibition of post-pituitary function. The former factor *a*) is probably important in all cases of stress and may bring about a significant diuresis, while *b*) diminished secretion of the antidiuretic, chloruretic hormone would also tend to produce the copious, dilute urine observed in our experiments. The acceleratory polyuria is readily inhibited or reversed by the injection of adequate doses of post-pituitary extract. This, however, does not necessarily implicate the pituitary gland in the reno-functional changes observed.

#### SUMMARY

The effects of acceleratory forces on renal function and pathology have been studied in the rat. The various degrees of exposure used were not evidently disturbing to the animal, although rather severe by human standards. During exposure to either positive or negative *g* forces, urinary secretion was suppressed. Following centrifugation the volume of urine excreted was increased above normal up to 40 per cent for five to six hours and its chloride concentration was reduced in comparison with non-accelerated controls. Acceleratory polyuria was reversed or inhibited by posterior pituitary extract. After repeated daily exposure to acceleratory forces over a period of about three weeks, albumin was present in the urine and significant pathological changes were found in the rat kidney. The effects are probably referable to anoxia brought about by reno-circulatory disturbances under high acceleratory forces. Cortico-adrenal and post-pituitary functions may be involved.

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# ELECTROPHRENIC RESPIRATION. III. MECHANISM OF THE INHIBITION OF SPONTANEOUS RESPIRATION<sup>1</sup>

STANLEY J. SARNOFF, JAMES L. WHITTENBERGER AND  
ESTHER HARDENBERGH

*From the Department of Physiology, Harvard School of Public Health*

BOSTON, MASSACHUSETTS

IN PREVIOUS communications it was demonstrated that artificial respiration could be produced by electrical stimulation of one or both phrenic nerves, with a series of impulses the voltage of which rose and fell in such a way as to produce effective diaphragmatic contraction and relaxation (1-3). It was found that smooth respiratory activity could be so induced and that the technique was capable of maintaining normal oxygen and carbon dioxide partial pressures in the arterial blood of the experimental animal and man in the absence of spontaneous respiration. The reserve of the technique was sufficient to permit hyperventilation and the production of alkalosis with stimulation of only one phrenic nerve.

An interesting observation made during the original study was that spontaneous respiration ceased within seconds after the onset of electrical artificial respiration. This observation was deemed to be of potential clinical importance, because of the manner in which patients not infrequently interfere with other types of artificial respiration. This is particularly true in patients with bulbar poliomyelitis. This report presents an examination of the mechanism by which spontaneous respiration is inhibited when electrical artificial respiration is started in the experimental animal.

## METHODS

Dogs of both sexes, weighing from 11 to 14 kg. were studied. Nembutal anesthesia, 30 mg. to 40 mg/kg. of body weight, was used. Electrophrenic respiration was applied by means of the apparatus previously described (2). Respiratory movements were recorded by means of a corrugated rubber tube placed around the lower part of the rib cage. Changes of pressure in the tube were registered on a direct-writing galvanometer by means of the electromanometer<sup>2</sup> in use in this laboratory. These pneumograms registered both diaphragmatic and intercostal activity but did not do so in a quantitative manner.

The partial pressures of oxygen and carbon dioxide in arterial blood were determined according to the technique of Riley (4). All determinations were done in duplicate immediately after the blood sample was drawn. Minute volumes were obtained with a spirometer. Vagus section was performed low in the neck.

The experiments described below were designed to yield data which might help answer five main questions: 1. Is all spontaneous respiration inhibited immediately after the onset of electrophrenic respiration? 2. If electrophrenic respiration does

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Received for publication September 27, 1948.

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis.

<sup>2</sup> Sanborn Company, Cambridge, Mass.

inhibit spontaneous respiration, is the suppression of chemical or neurogenic origin? 3. If the inhibition is of a reflex nature, what is the afferent pathway over which the reflex travels? 4. After interruption of the afferent pathway, can spontaneous respiration still be inhibited by overventilation? 5. Is the reflex inhibition of spontaneous respiration vigorous enough to inhibit spontaneous respiration in the presence of a strong chemical respiratory stimulus?

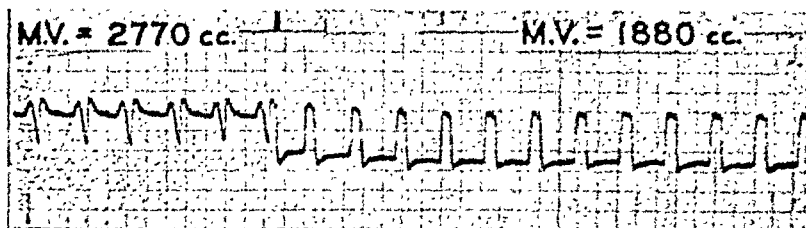


Fig. 1. PNEUMOGRAM OF ONSET OF ELECTROPHRENIC RESPIRATION. Artificial respiration started at signal. M.V. = min. vol. before and during electrophrenic respiration. Larger deflection during electrophrenic respiration does not indicate deeper respiration but does indicate stronger diaphragmatic contraction. Downward deflection registers inspiration and the same is true for subsequent figures. Paper speed is 2.4 mm/sec.

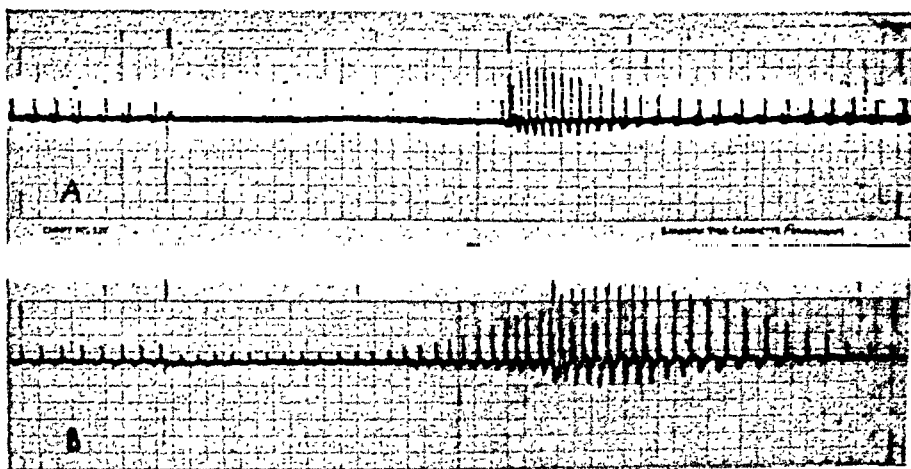


Fig. 2. SUSTAINED DIAPHRAGMATIC CONTRACTION: *A*, vagi intact; *B*, vagi cut. Sudden continuous and sustained contraction of diaphragm was started at the first signal and discontinued at the second in both tracings. In tracing *A*, before the vagi were cut, all effective intercostal respiration was inhibited by keeping the diaphragm contracted. In tracing *B*, after vagotomy, the intercostal rhythm was not suppressed by the same degree of sustained diaphragmatic contraction.

## RESULTS

1. *Immediate inhibition of spontaneous respiration.* Figure 1 is an example of the immediate cessation of spontaneous respiratory effort which occurred in a dog immediately after the onset of electrophrenic respiration. The pneumogram pattern shows a machine-like regularity during electrophrenic respiration and contains no evidence of interference by spontaneous respiratory efforts. This cessation of spontaneous respiration occurred despite the fact that the respiratory rate was unchanged and the minute volume was lower than that observed before the onset of electrophrenic respiration. That spontaneous breathing is really inhibited, and not

just masked by electrophrenic respiration, is borne out by the fact that, when normal or greater than normal minute volumes are maintained for only a fraction of a minute by the electrophrenic respirator, a brief period of apnea follows the cessation of stimulation. A similar observation was made in man (3).

2. *Neurogenic inhibition of breathing by sustained diaphragmatic contraction.* In figure 2A can be seen the effect of continuous electrically sustained diaphragmatic contraction. No spontaneous respiratory effort was made until just before the stimulation stopped. During a period of similar diaphragmatic contraction performed a short while before the taking of the tracing in figure 2A, the arterial partial pressure of  $\text{CO}_2$  changed from 37 mm. Hg before stimulation to 41 mm. Hg 50 seconds after the application of the stimulus. Oxygen partial pressure changed from 84 to 60

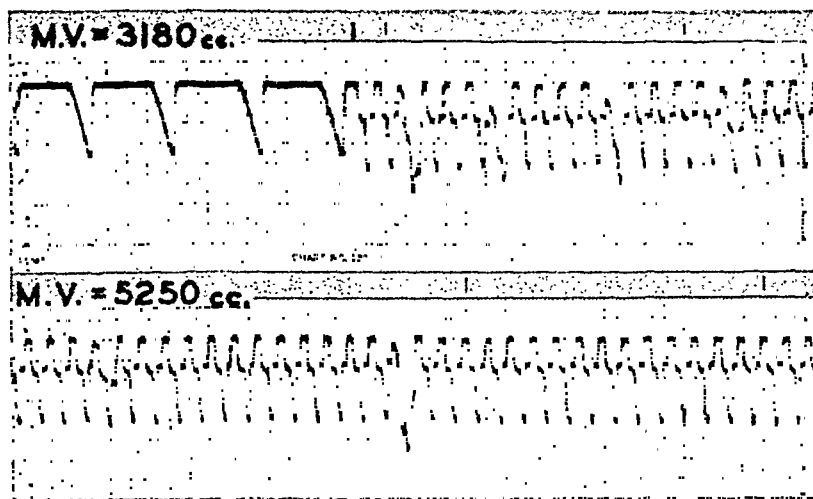


Fig. 3. OVERVENTILATION BY ELECTROPHRENIC RESPIRATION after vagi have been cut. Electrophrenic respiration started at the signal. M.V. = min. vol. before and after the onset of electrophrenic respiration. Tracings are continuous. No further irregularities of the respiratory pattern occurred after the one seen in the middle of the lower tracing.

mm. Hg. Despite this rise in  $\text{CO}_2$  and fall in  $\text{O}_2$ , no spontaneous respiratory effort was made during this 50-second period.

3. *Afferent pathway of the inhibitory reflex.* The tracing in figure 2B, taken 12 minutes later, shows the result of the same procedure 7 minutes after section of both vagus nerves. It can be seen that spontaneous respiratory movements continued at the same rate throughout the period of stimulation. They are, however, of smaller amplitude than before stimulation, since the electrical splinting of the diaphragm confines respiratory activity to the intercostal muscles during the stimulus period.

4. *Effect of overventilation after vagotomy.* In another experiment, with sectioned vagi, after setting the electrophrenic respirator for an appreciably higher respiratory rate and minute volume than the dog's spontaneous rate and minute volume, electrophrenic respiration was started. Figure 3 shows the result. It can be seen that spontaneous respiration was not immediately inhibited and subsided only after a period of overventilation had occurred.

5. *Reflex inhibition of respiration during inhalation of  $\text{CO}_2$ .* A dog was made to breathe 7 per cent carbon dioxide in 93 per cent oxygen. The electrophrenic respi-

rator was set for a rapid deep respiration and started 12 minutes after the dog had begun breathing the above gas mixture. Figure 4 shows the result. It can be seen that the respirator assumed immediate control and inhibited spontaneous respiration while a high level of carbon dioxide (71 mm. Hg) was present in the arterial blood. The presence of barbiturate anesthesia may have decreased the sensitivity of the respiratory center to carbon dioxide. It cannot be doubted, however, that reflex suppression of spontaneous respiration was an important element, for, while still breathing 7 per cent  $\text{CO}_2$ , after cutting the vagi, definite interference with the respirator's control occurred, as is evident from figure 5. This interference with the respirator's control persisted for the duration of the observation period (15 min.).

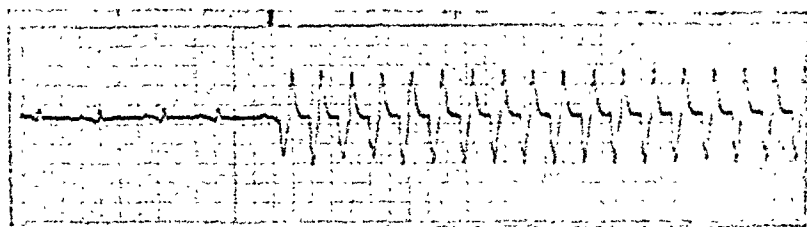


Fig. 4. ELECTROPHRENIC RESPIRATION during administration of 7%  $\text{CO}_2$ . The above tracing was taken 12 min. after the dog was made to breathe 7%  $\text{CO}_2$  in 93%  $\text{O}_2$ . During this period the arterial blood  $\text{CO}_2$  partial pressure was 71 mm.Hg. Rapid, deep, electrophrenic respiration was begun at the signal and assumed control of respiration.

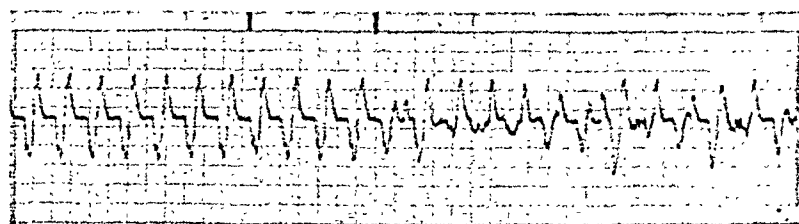


Fig. 5. ELECTROPHRENIC RESPIRATION during administration of 7%  $\text{CO}_2$  before and after vagus section. The same preparation as in fig. 4. Dog still breathing 7%  $\text{CO}_2$  in 93%  $\text{O}_2$  and is still under the control of the electrophrenic respirator at the beginning of the record. Left vagus cut at the first signal and right vagus cut at the second signal. Interference with the electrophrenic control of respiration by spontaneous respiratory motions is apparent immediately after vagotomy.

This experiment also confirmed the fact that the initial immediate suppression of respiration in the previous experiments (fig. 1) was not due to the excessive elimination of carbon dioxide.

#### DISCUSSION

The data presented demonstrate that spontaneous respiration ceases immediately after the onset of electrophrenic respiration. In previous experiments on the dog, cat, rabbit and monkey, this phenomenon was also observed. That this inhibition has two components has been demonstrated above. The first of these is a reflex set up by diaphragmatic contraction. At least a portion of the reflex has its afferent pathway in the vagus nerves. For with intact vagi, a sustained diaphragmatic contraction reflexly inhibits respiration even though the arterial oxygen falls and carbon

dioxide rises (fig. 2A). After section of the vagi this phenomenon no longer occurs (fig. 2B).

The second component in the inhibition of spontaneous respiration is the change in blood chemistry brought about by electrophrenic respiration, if the respiration thus induced is sufficient to overventilate the animal. That this component can also be effective in inhibiting respiration in the absence of the reflex component is demonstrated by figure 3.

The potency of the reflex inhibition of spontaneous respiration by electrophrenic respiration can be deduced from those experiments in which, despite an extreme level of carbon dioxide present in the blood, spontaneous respiration did not occur while vigorous electrophrenic respiration was in effect (fig. 4).

We have been vague concerning the precise location of the sense organs initiating the impulses that inhibit spontaneous respiration when electrophrenic respiration is applied. It is, of course, likely that the Hering-Breuer reflex, as it is customarily interpreted (5), plays a part in the reflex inhibition of respiration as described above. However, certain proprioceptive sense organs other than those which send impulses up the vagus nerve may prove significant and to ascribe the effect solely to stretching of the pulmonary parenchyma may be to overlook certain other significant contributing afferents. The fact that forceful endotracheal insufflation does not cause a comparable immediate reflex inhibition of respiration indicates that simple stretch of the pulmonary parenchyma is not the only factor involved in the reflex inhibition described in the above experiments. Simple stretch of the pulmonary ligament has, under certain conditions, been found to inhibit inspiration and to initiate a forceful sustained expiratory effort. This matter is under investigation at the present time.

#### SUMMARY

The inhibition of spontaneous respiration by electrophrenic respiration has two components. The first is neurogenic and the second chemical. The first, reflex in nature, consists, in part, of impulses that travel up the vagus nerve during the period of diaphragmatic contraction. This reflex disappears after sectioning the vagi. The reflex is immediate if the initial diaphragmatic contraction is adequate. In addition to the reflex inhibition, spontaneous respiration can be inhibited by providing electrophrenically induced overventilation. The potency of the neurogenic inhibition has been demonstrated by the fact that it can inhibit spontaneous respiration in the presence of a vigorous chemical stimulus to respiration, namely a high carbon dioxide partial pressure in the arterial blood.

The authors wish to express appreciation to Mrs. Harriet A. Kriete for the performance of blood gas analyses, and to Mr. Philip Waithe for his technical assistance in the experiments.

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# FLUID SHIFTS IN ANIMALS DURING PRESSURE BREATHING<sup>1</sup>

CHESTER HYMAN AND JOSEPH GOODMAN

*From the Department of Physiology, University of Southern California Medical School*

LOS ANGELES, CALIFORNIA

THE use of high positive pressure respiration, combined with adequate counter-pressurization presents a novel environment for man and animals. Much of the physiology of pressure breathing has recently been reviewed by Barach *et al.* (1). Work in our laboratory has been directed towards the specific problems of fluid dynamics during exposure to high positive pressure respiration. The first of these studies by Henry *et al.* (2) is concerned with the measurement of fluid loss in men under these conditions. We have made a parallel study of the circulatory effects of high pressure breathing with partial protection in cats. Interest was focused on the rate and amount of fluid loss as indicated by hemoconcentration, the extent of protein leakage from the circulation and the kinetics of disappearance of an injected dye.

The methods developed for this study are applicable to use in quantitative investigations of vascular filtration. Unlike the venous occlusion technique, the pressures attainable are theoretically unlimited, since pressure breathing theoretically increases both the venous and the arterial pressures. It is therefore possible to achieve any reasonable pressure level in the capillaries of the exposed limbs and thus provide extremely high filtration pressures. Since all four limbs (or any number of limbs) may be left unprotected, the area for filtration is considerably greater than in the case of occlusion of a single extremity and therefore significant fractions of the total circulating volume may be driven from the circulation.

## METHODS

Cats weighing between 2.4 and 4.7 kg. were used as experimental animals. They were intravenously anesthetized with pentobarbital (ca. 35 gm/kg. body wt.) and were given an equal maintenance dose subcutaneously. The animals were then prepared by exposure of the large blood vessels in the femoral region and the insertion of sub-dermal electrocardiogram leads.

Protection was provided by counter-pressurization of the thoracic and abdominal areas with an air bladder and non-distensible vest. The bladder was made of two thicknesses of vinylized nylon cloth, with appropriate openings for all four limbs, and an opening along the mid-dorsal line. (See insert, fig. 1). The stitched edges were made air-tight by sealing with rubber cement. Pressurizing gas was conducted into the bladder through an aluminum bushing, bolted and cemented into the external layer only. The jacket design closely followed the same pattern, except that it was made of a single layer of 'Berger' cloth and was provided with a tongue and

Received for publication September 27, 1948.

<sup>1</sup>This study is based on work performed under Contract No. W-33-038-ac-14711 between the University of Southern California and the U. S. Air Force Air Materiel Command.

eyelets along the dorsal opening to provide an adjustable closure by lacing. In addition, suitable straps and buckles were sewed to the anterior end to give a better fit around the neck and as points of attachment for the helmet. This system provides protection for all of the body with the exception of the head and the four limbs.

In the earlier studies tracheal cannulation was used and partial protection for the head was achieved by tight binding with elastic bandages. This technique was not entirely satisfactory, since it required surgical manipulations in the neck and there was no assurance that counter-pressurization of the head had been adequate. The system was therefore modified to include a lucite helmet, which was large enough to encase the entire head and which was sealed by means of a vinylized nylon 'skirt' which could be slipped between the air-bladder and the vest at the neck (cf. fig. 1).

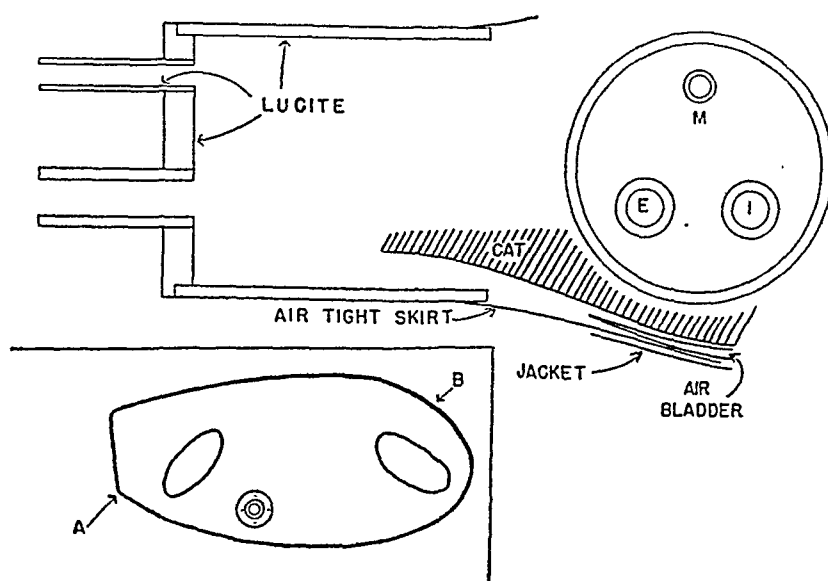


Fig. 1. DETAILS OF PRESSURE BREATHING HELMET and counter-pressurization system. Method for making air tight joint between helmet and cat is shown in lower right. *Insert:* pattern, much reduced, for the counter-pressurization system. *E*, connection to expiratory system; *I*, connection to inspiratory system; *M*, connection to manometer.

This system provided satisfactory counter-pressurization for the head and neck and in extreme trials showed no important leaks at pressures as high as 180 mm. Hg.

The pressure breathing system consisted of two diaphragm-type pressure regulating valves, connected on their high pressure side to a source of compressed air or oxygen. The output of one regulator ( $R_i$  of fig. 2) was connected directly with a solenoid valve ( $V_i$ ); the output of the other regulator ( $R_e$ ) was connected to the loading port of a modified Linde mask exhalation valve ( $L$ ). The exhalation port of the Linde valve was connected to a second solenoid valve ( $V_e$ ). These electrically operated valves were then connected either to the tracheal cannula or to the pressure helmet. The protective counter-pressurization bladder was connected to the respiratory circuit at some point between the two solenoid valves. The timing and alternation of inspiration and expiration was achieved by alternately opening the two solenoid valves. A suitable telechron driven cam ( $S$ ) operated a switching system to give a respiratory rate of 60 (or in later experiments 30) cycles/min., with approximately two-thirds of each cycle in inspiration and one-third in expiration.

The arrangement of the several pressure and electrical circuits are schematically represented in figure 2.

*General Procedure.* Immediately after completing the preparation, a single control sample was taken from the femoral artery. Soon thereafter about 0.25 mg. of the dye T-1824/kg. body weight was injected into the femoral vein. At precisely measured intervals of about 15 to 30 minutes after the dye injection, further arterial blood samples were taken. Thirty to 70 minutes later the animal was disconnected from the pressure system and samples were taken during the subsequent 90 minutes.

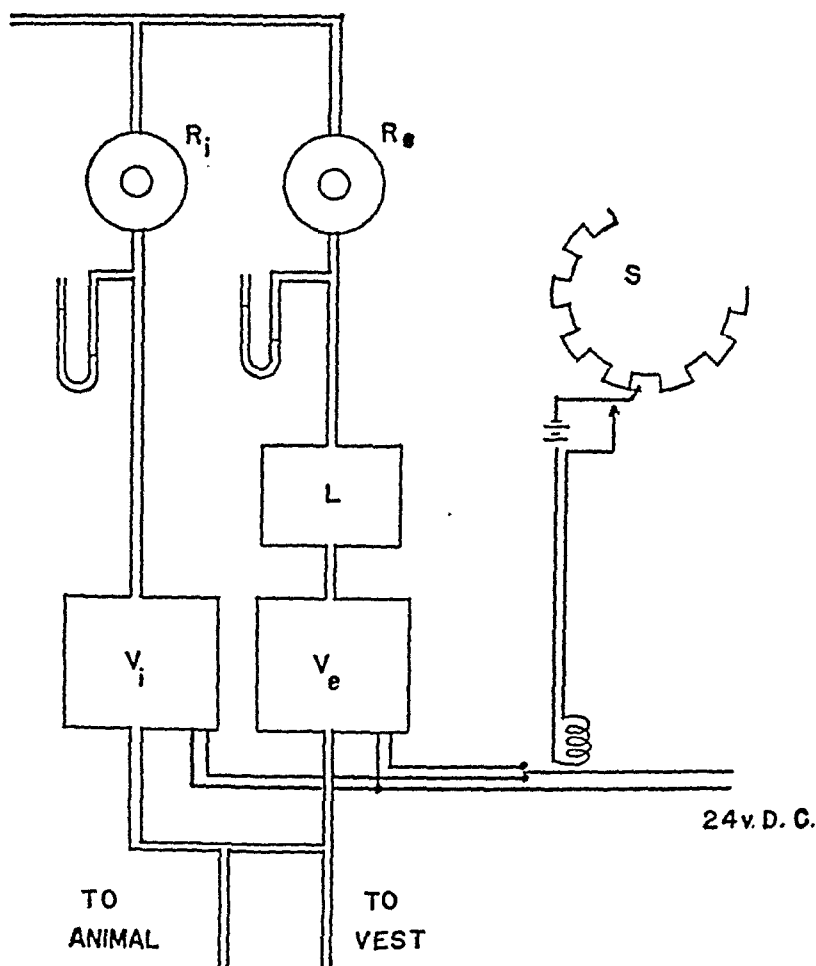


Fig. 2. SCHEMATIC REPRESENTATION of the pressure breathing system.

*Analytical Techniques.* Fluid shifts were determined from four sets of data: hematocrit, hemoglobin, plasma protein concentration and dilution of the injected dye. For each sample, one ml. of blood was taken into a syringe containing 1.6 per cent sodium oxalate. The amounts of oxalate and of blood were determined gravimetrically and were approximately in a ratio of 1:5. After thorough mixing, ca. 0.8 cc. was delivered into a Wintrobe hematocrit tube. The remainder, 0.3 to 0.4 cc., was utilized for hemoglobin determinations by the colorimetric acid hematin technique. The hematocrit values were determined directly by the method of Wintrobe (3). The supernatant plasma in the hematocrit tubes was removed by pipette. Exactly 0.2 ml. of this plasma was diluted with 1.0 ml. of 0.9 per cent

saline. The dye concentrate in this diluted plasma was then determined with a Beckman quartz spectrophotometer. Plasma protein concentrations were determined by the falling drop method of Barbour and Hamilton (4) on another aliquot of the supernatant fluid in the hematocrit tube. All data were corrected for dilution of oxalate and saline.

#### RESULTS AND DISCUSSION

A total of 48 cats were used in this study. Of these, 7 animals were exposed to pressure breathing in the helmet described, while 37 experiments were performed with tracheal cannulation and the remaining animals served as controls for the sampling techniques. Of the 44 animals exposed to pressure breathing, 30 survived the entire experiment, including a post-experimental period of 30 to 60 minutes. The fatalities could usually be attributed to accidents or to some obvious fault in the technique. Since adequate data were not obtained in all cases, the number of animals on which results are cited will be less than these totals.

No significant differences were noted between the data obtained with the pressure helmet and those obtained in the experiments with tracheal cannulation, therefore all of the results are treated together in table 1. The percentage of fluid loss was calculated from the average prepressure breathing values and the highest values of hematocrit or hemoglobin obtained during the stress. The calculations were made on the assumption of a constant volume of circulating erythrocytes throughout the experiment. Plasma protein leakage was judged qualitatively from the relationship between the hematocrit shift and the plasma protein concentration shift during the same period. The ratio of initial hematocrit to initial plasma protein level is presented as a rough index of the condition of the animal at the beginning of the experiment. While a low hematocrit might result from over-hydration, a low hematocrit:plasma protein ratio would suggest a real anemia. The respiratory pressure given is the arithmetic mean of the inspiratory and expiratory pressures used. This value probably is somewhat smaller than the true value, since the duration of the inspiratory (high pressure) was in all cases greater than the expiratory phase. The inspiratory pressures ranged from 22 to 120 mm. Hg, while expiratory pressures were between 40 and 60 mm. Hg.

In spite of the wide variation in the individual values, it will be noted that there is a tendency towards greater fluid loss in animals subjected to the higher pressures (*Group B* as compared with *Group A*). The animals in *Group C* are all characterized by a somewhat lower hematocrit:protein ratio, and showed abnormally high fluid loss, with a consistent tendency for protein leakage.

The kinetics of the fluid shifts may be judged from figures 3 and 4. In these figures the hematocrit, hemoglobin and plasma protein values are plotted as percentage of the control values at the times of the several samples. Figure 3, representing data on a cat with a high hematocrit:protein control ratio, shows a moderate fluid loss and negligible protein leakage. The data in figure 4 were obtained on a cat with a low ratio (representative of *Group C*) and show a marked fluid loss with a strong tendency for protein leakage. The curves representing the disappearance of dye from the circulation showed no important deviations from the control when properly corrected for the fluid shifts.

In general, our results indicate 1) a fluid loss, the magnitude of which is, at least in part, dependent on the mean pressure employed in the respiratory system;

TABLE 1. FLUID LOSS FROM THE CIRCULATION OF CATS DURING PRESSURE BREATHING

ANIMAL NO.	WEIGHT	FLUID LOSS <sup>1</sup>		PROTEIN <sup>2</sup> LEAKAGE	MEAN RESP. <sup>3</sup> PRESSURE	HCRT/PROTEIN <sup>4</sup>
		HCRT	Hb			
		per 100 cc.				
	kg.				mm. Hg	
Group A						
31	2.5	3.8	5.5	±	33	70.8
34	2.5	5.9	6.4	+	35	78.4
35	3.4	1.0	2.7	?	37.5	
36	3.3	8.3	8.6	—	37.5	62.7
Av.....		4.75	5.8		35.7	70.9
Group B						
6	2.9	11.3	13.6	?	48	
7	3.1	16.3		?	48	
8	2.4		15.4	?	47	
11	3.1	11.8	14.2	+	48	58.0
13	2.9	7.8	12.4	?	43	
14	2.9	12.9	13.3	—	43	65.7
16	2.8	11.3	12.2	—	42	62.4
21	?	12.7		±	42	63.4
24	2.4	9.4	9.6	?	42	
41	2.3	10.3		±	47.5	84.6
42	2.3	10.5		—	47.5	77.6
43	2.3	9.2	8.5	—	41.5	55.3
45	3.4	3.5	4.4	—	45	55.7
Av.....		10.6	8.8		45	65.4
Group C						
15	3.5	22.5	20.7	+	43	53.9
17	3.5	30.5	31.2	+	43	52.0
20	?	17.9	16.1	±	42	56.1
32	2.8	32.7	28.9	+	35	46.8
37	4.7	28.3	32.7	+	37.5	39.4
Av.....		26.4	25.9		40.1	49.7

<sup>1</sup> Fluid loss was calculated from the av. pre-experimental hematocrit or hemoglobin values and the highest value observed during pressure breathing. The peak hematocrit and hemoglobin values were usually obtained in the same blood sample. More than 3/4 of the fluid was lost in 20 mins. after beginning pressure breathing.

<sup>2</sup> Protein leakage was judged as described in the text. A + indicates marked leakage; ± intermediate; and — indicates no leakage.

<sup>3</sup> The arithmetic mean of the inspiratory and expiratory pressures imposed.

<sup>4</sup> Ratio of the control hematocrit and plasma protein levels.

2) no consistent increase in protein leakage as a consequence of the pressure breathing per se; and 3) no fundamental change in the dye-disappearance curves. These re-

sults are concordant with the theoretical predictions based on increased capillary hydrostatic pressure. They suggest that the simple distention of the smaller blood vessels evoked by the procedures used are not of sufficient magnitude to alter their normal impermeability to proteins.

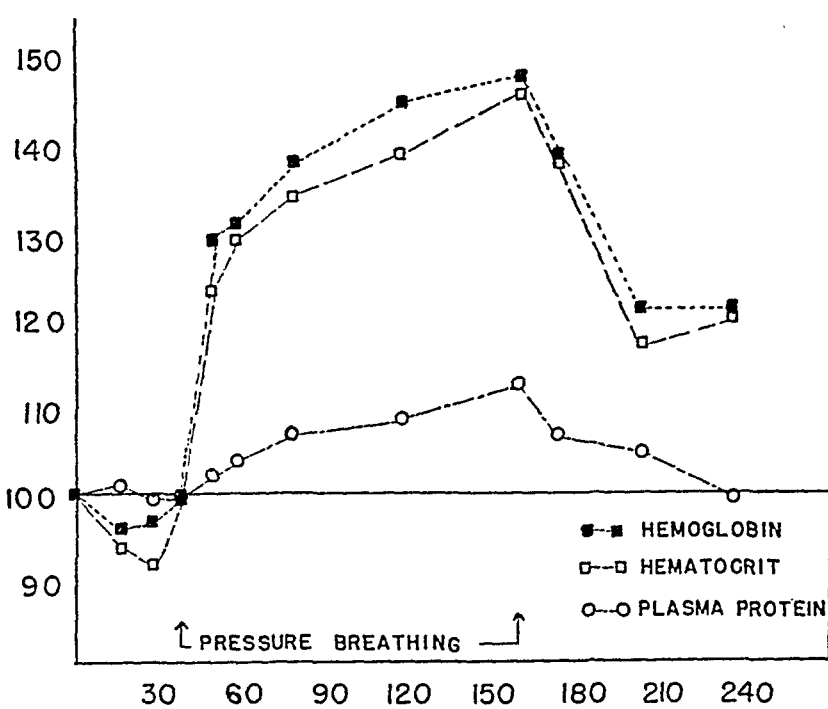


Fig. 3. CHANGES IN HEMATOCRIT, hemoglobin and plasma protein values during an exposure to pressure breathing. Time in minutes, other values as percentage control. Animal which showed marked fluid loss and much protein leakage.

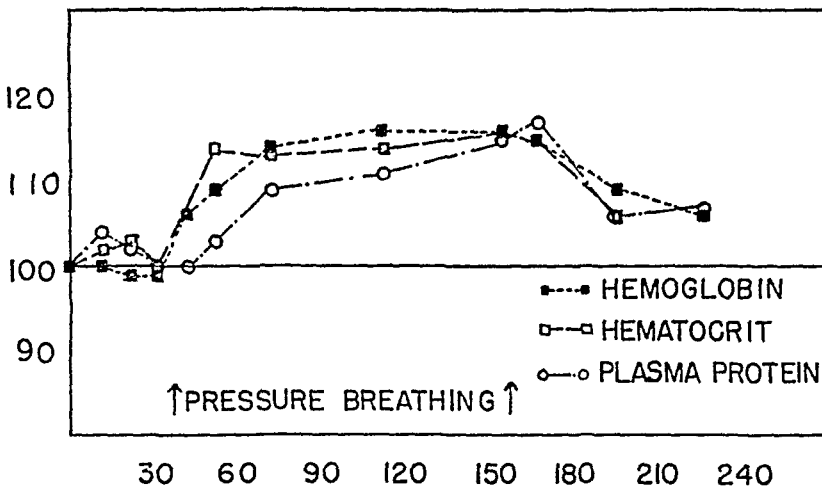


Fig. 4. CHANGES IN HEMATOCRIT, hemoglobin and plasma protein values during an exposure to pressure breathing. Time in minutes, other values as percentage control. Animal with typical fluid loss and no protein leakage.

During pressure breathing, complete counter-pressurization would theoretically protect the subject against all the cardiovascular and mechanical disturbances which otherwise would result from the increased respiratory pressures. With limited counter-pressurization, however, there is opportunity for significant decrease in

effective circulating blood volume due to congestion, pooling and filtration of fluid in the unprotected areas of the body. These mechanisms for fluid loss are merely extensions of the phenomena encountered with pressure breathing at lower levels where counter-pressurization is not employed.

The findings in the present study confirm the observations of Henry, *et al.*, made on man during conscious pressure breathing and extend them to anesthetized animals. Certain of the results, however, are specific for the preparation used and should be considered here.

The hematocrit:protein ratio may reflect the general state of the animal, or may more specifically measure the absolute anemia of the cats. In either event, the ratio serves as an empirical index for predicting which animals will behave in an atypical fashion during pressure breathing. It is unlikely that the anemia itself is of sufficient magnitude to lead to an anemic anoxia with a consequent increase in capillary permeability. Henry, Goodman and Meehan (5) have shown that extremely low oxygen levels are required to produce changes in capillary permeability. These studies again demonstrate the rapidity with which fluid may be displaced from the circulation by purely hydrostatic factors. Shifts of more than 10 per cent of the total blood volume in less than 30 minutes of pressure breathing have frequently been observed.

#### SUMMARY

A method has been developed for exposing anesthetized animals to high positive pressure respiration, using a counter-pressurization system and a simple helmet. Animals exposed to pressure breathing show a rapid loss of fluid from the circulation which is roughly proportional to the mean respiratory pressure employed. Protein leakage was inconstantly observed in animals exposed to pressure breathing.

We are pleased to acknowledge the technical assistance of D. Gordon, R. Frankel, A. Klain and Martha Mill in these studies. Doctors J. P. Henry and D. R. Drury originally suggested the problem and cooperated with us throughout the study.

These experiments were performed in laboratories generously provided by the Allan G. Hancock Foundation.

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# NATURE OF THE PRECORDIAL ELECTROCARDIOGRAM<sup>1</sup>

L. H. NAHUM AND H. E. HOFF<sup>2</sup>

*From the Laboratory of Physiology, Yale University School of Medicine*

NEW HAVEN, CONNECTICUT

*and the Department of Physiology, McGill University*

MONTREAL, CANADA

THE precordial electrocardiogram has assumed importance in clinical electrocardiography because in certain instances it shows changes not apparent in records from the limb leads, and on this ground alone it would be useful to have more complete information concerning its origin. In addition to the simple recognition of normal and abnormal complexes, the lead has been credited with a special significance in regard to electrocardiographic theory. The essential feature of its special significance lies in the assumption that unipolar precordial leads "are in reality semi-direct leads from the anterior ventricular surface, capable within certain limits of serving the same purposes as direct leads from the ventral wall of the exposed heart" (1, p. 27).

Viewed in this light the lead becomes largely a reflection of physiological events transpiring within the myocardium lying directly beneath the exploring electrode, with lesser contributions from other regions made possible by the alterations they cause in the potential of the cavity, which are thereby transmitted to the exploring electrode.

While therefore unique properties of a theoretical nature are invoked for precordial leads, in practice the records taken with such a lead are interpreted as representing the full sequence of excitation and recovery of all parts of the ventricle beneath the recording electrode. In picturing this chain of events, resort is had to the conventional dipole hypothesis. The upstroke of R is taken to represent the outward passage of the wave of excitation from endocardium to epicardium directly beneath the chest electrode, producing, according to theory, an electrical change which can be represented by a dipole with the positive charge oriented toward the epicardium. The downstroke is inscribed when the full thickness of the ventricle under the electrode has become excited and local differences of potential between endocardium and epicardium disappear. Q-waves are said to appear when some part of the endocardial myocardium at a distance from the chest electrode is excited in advance of the region under the electrode. Such activity would produce a similar array of dipoles in that region, and, since normal excitation is considered to progress in all parts of the heart from endocardium to epicardium, the cavity of the ventricle would become negative, and this negativity, being transmitted to the chest electrode, would produce the downstroke of a Q-wave. In a like manner an S-wave would appear if an impulse were still traveling in an outward direction in any part of the ventricle after the impulse had already broken through to the surface underneath the chest electrode.

The T-wave is supposed similarly to arise mainly from differences in the time of onset and rate of repolarization between endocardial and epicardial muscle layers lying beneath the exploring elec-

Received for publication October 11, 1948.

<sup>1</sup> This work was supported by grants from the Cooper Fund, Faculty of Medicine, McGill University, and the Fluid Research Fund, Yale University School of Medicine.

<sup>2</sup> Present address: Baylor University College of Medicine, Houston, Texas.



trode, although similar differences in the repolarization of endocardial and epicardial layers at distant points would also affect the T-wave because of the influence they would exert on the potential of the cavity.

When this hypothesis is used to interpret complexes of unknown origin, it becomes apparent immediately that two alternative explanations are always possible for each portion of the ventricular complex. Thus a Q-wave might represent the outward travel of the excitation process at a point at a distance from the chest electrode or the inward travel of an impulse beneath the precordial electrode. The R-wave could represent the inward travel of a wave of excitation distant from the chest electrode, or an outward traveling wave beneath it. Alternative origins for the S-wave are also possible. It may be granted that some of these alternatives are not likely to occur if one assumes a certain normal sequence of excitation, but they become as likely as any of the others in cases of conduction defects and abnormal rhythms.

The alternatives regarding the T-wave are not however resolved by recourse to assumptions regarding the normal distribution of the cardiac impulse. To explain an upright T-wave it is assumed either that the epicardium beneath the exploring electrode begins repolarization ahead of the subjacent endocardial lamellae, or that the endocardium at a distance begins its repolarization before the overlying epicardium, and there is no collateral evidence to indicate which if any of these is the more likely. The localizing significance of RS-T segment deviations is equally unclear. It has been claimed that lesions damaging the endocardium beneath the precordial electrode produce downward displacement of the RS-T segment, while it is known that similarly situated epicardial lesions cause an upward deviation (2). Thus it would be impossible to determine from an upward displacement of the RS-T segment whether the lesion is in the epicardium directly beneath the electrode or in the endocardium at a distance.

Some direct experimental evidence bearing on these questions is already at hand (3). An R-wave could never be produced by stimulation of the endocardium underneath the chest electrode. Neither could Q- or S-waves be produced by stimulating endocardial areas of the heart at a distance from the electrode. Quite to the contrary, R-waves could only be produced by stimulation of distant areas, and Q- and S-waves were only produced by stimulation of either endocardium or epicardium in the region of the chest electrode (3).

A comparable study of the T-wave in which localized endocardial or epicardial cooling or warming was employed showed that it was not possible to differentiate between the effects of treatment of epicardial and immediately subjacent endocardial layers of the myocardium (4). The only way to invert the T-wave was to delay repolarization of the muscle underneath the chest electrode whether endocardial or epicardial, or to hasten repolarization of distant regions, whether endocardial or epicardial. Similarly increase in amplitude of T could be produced only by hastening repolarization of epicardial or underlying endocardial regions under the electrode or by delaying repolarization of distal regions be they endocardial or epicardial (4).

The implication of this evidence is that the precordial electrocardiogram arises from the interplay of but two opposing forces, one representing the excitation of the proximal region under the chest electrode and the other the excitation of certain regions distal to the chest electrode. Quite without regard to the pathway by which excitation arrives, activation of the proximal zone tends to produce a downward movement in the record, and depending on the sequence of events in the areas involved, produces respectively the downstrokes of Q, R and S. Excitation of distal areas is responsible for the upward movement in the record, whether toward the diastolic baseline as in Q and S, or away from it as in R. These functions are exclusive; downward movements can be produced only by 'proximal' excitation, upward movements only by 'distal' excitation. Stated in another way the evidence thus far produced supports the view that the precordial electrocardiogram is derived from the interference between potentials resulting from excitation in the proximal zone which tend to move the beam downward and potentials derived from excitation of distal regions whose effect is to move the beam upward.

The experiments reported here were devised to extend the observations outlined above, and to delimit in some detail the topographical extent of the distal and proximal zones as recorded in the several precordial leads in the dog's heart.

## METHODS

Fifteen dogs were employed, anesthetized deeply with Nembutal or Dial. The heart was exposed by incision along the lower border of the left pectoral muscle and

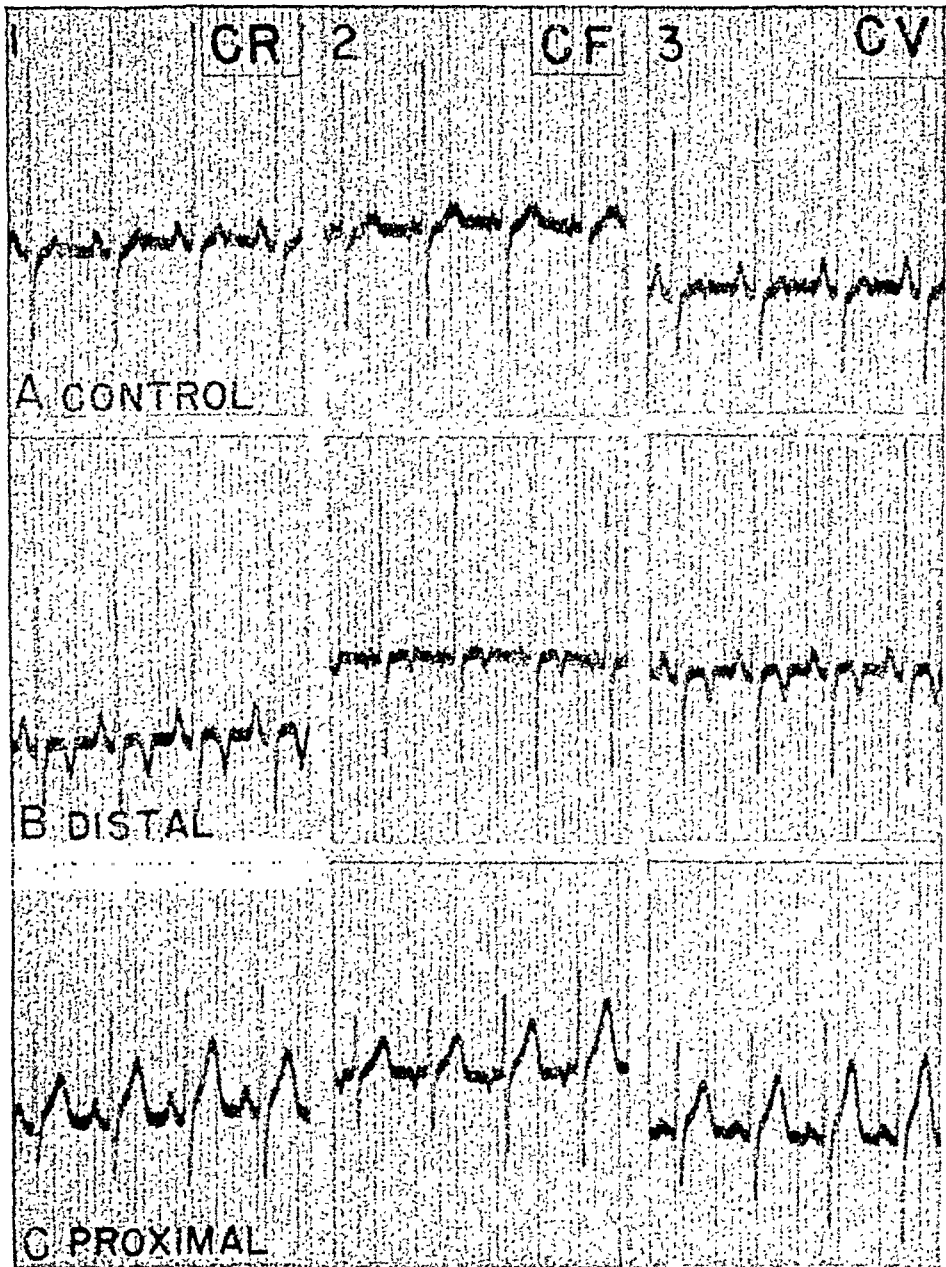


Fig. 1. Dog, Sept. 1, 1944. Dial anesthesia. Right apical precordial lead. 1, CR; 2, CF; 3, CV. A. Control. B. Warming left base (distal zone). T wave becomes sharply inverted. C. Warming right apex (proximal zone). T wave sharply upright.

removal of the sternal third of the fifth rib. Lungs were inflated fully, the chest wall was closed and spontaneous respiration reestablished before records were taken, except in experiments with warming or cooling, where a completely air-tight closure could not be effected. CF, CR and CV leads were employed with the chest electrode

opposite the left apex on the left side of the chest and the right apex on the right side of the chest. A Sanborn Tribeam was employed at standard sensitivity. The entire external surface of the heart was explored to determine *a*) the influence on the S-T segment of localized application of 0.1 M KCl solution by means of squares of blotting paper soaked in the solution, *b*) the influence of local warming and cooling

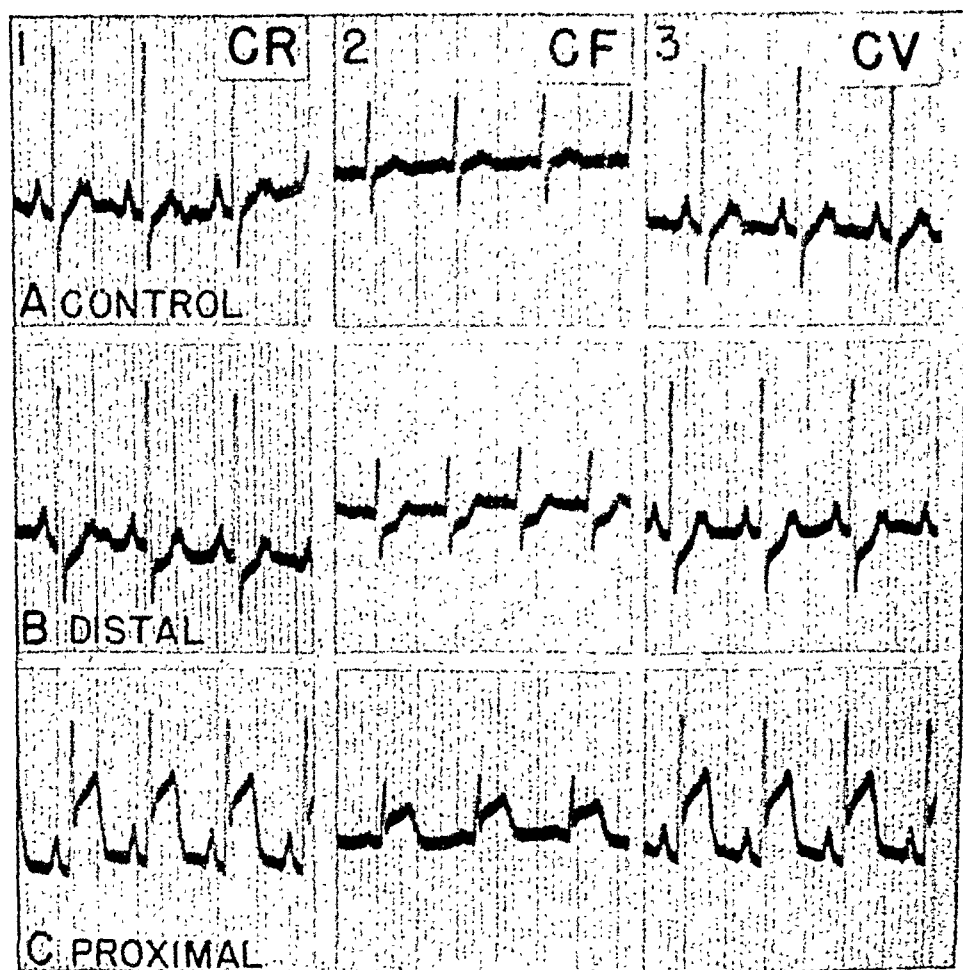


Fig. 2. Doc, same as fig. 1. Right apical lead. *A*. Control. 1, CR; 2, CF; 3, CV. *B*. Isotonic KCl applied to right base (distal zone). S-T segment depressed in each lead. *C*. Isotonic KCl applied to right apex (proximal zone). S-T segment elevated.

of the myocardium on the T-wave and *c*) the configuration of extrasystoles elicited from the regions of the heart studied in (*a*) and (*b*).

#### RESULTS

All three modes of investigation yielded comparable results: 1) in each case an area could be mapped out under the chest electrode which, when warmed, damaged, or stimulated, produced characteristic 'proximal' electrocardiographic changes. 2) An area of considerably greater extent involving regions of the heart distant from the chest electrode could be demarcated which gave rise to alterations of opposite

nature to the 'proximal' changes; these can be called 'distal' effects. 3) Between these regions was found an intermediate zone which was largely silent or neutral.

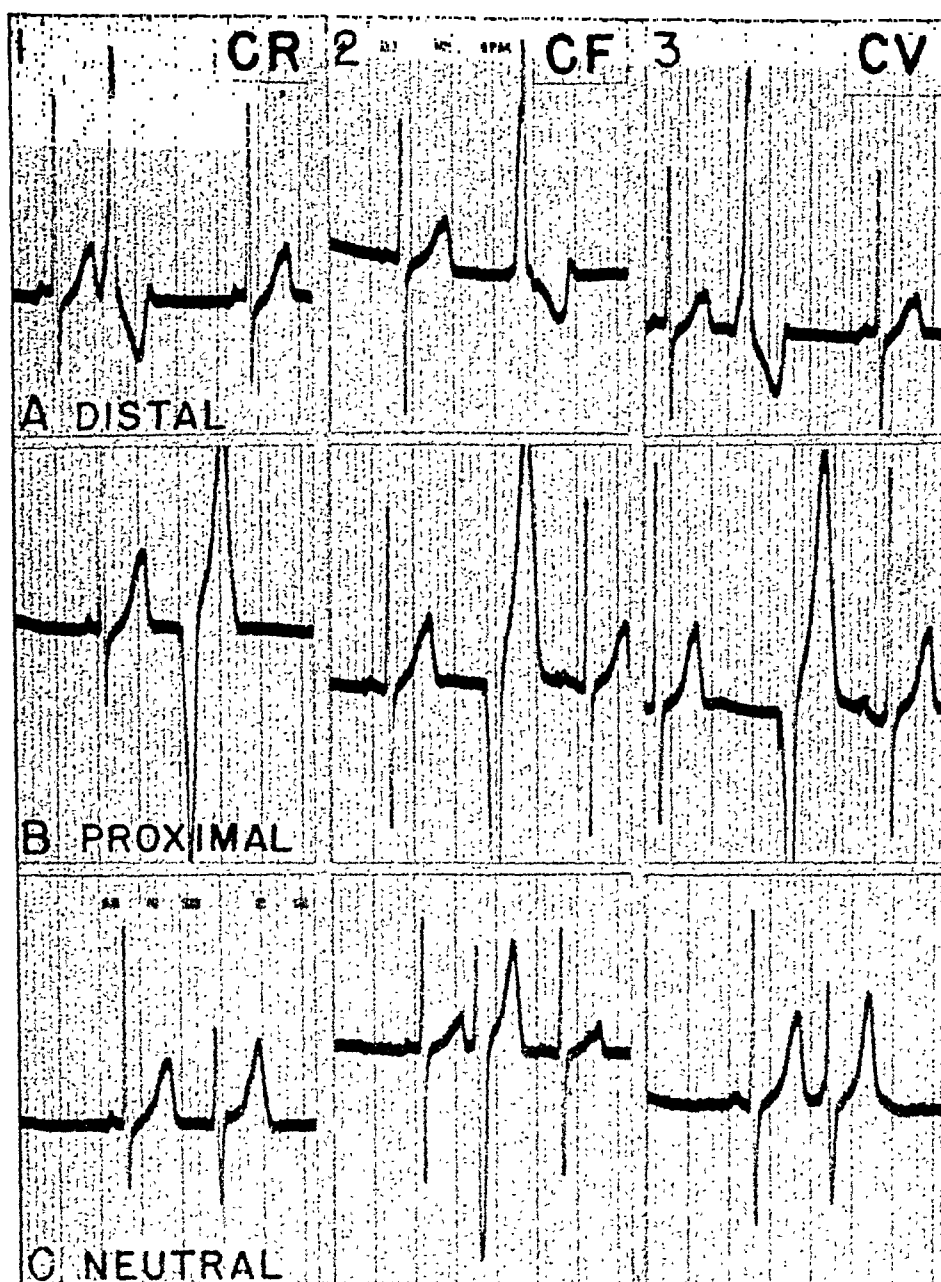


Fig. 3. Dog, August 31, 1944. Dial anesthesia. Right apical lead. A. Extrasystoles in CR, CF, and CV leads elicited by threshold stimulation of a point in the anterior left base (distal zone). Initial beam movement is upward. B. Extrasystoles elicited by threshold stimulation of a point at the right apex at the septum (proximal zone). Initial beam movement is downward. C. Extrasystoles elicited by threshold stimulation points in the neutral zone of each lead. 1. left lateral apex; 2. right base; 3. posterior septum midway between base and apex. In each case a diphasic complex results.

Localized warming of the myocardium under the chest electrode caused a progressive elevation of the T-wave leading to the development of exaggerated positive

T-waves. These reached a maximum height after one or two minutes and thereafter maintained that amplitude for as long as warming persisted. Warming distal regions produced, on the contrary, a progressive diminution in height of the T-wave

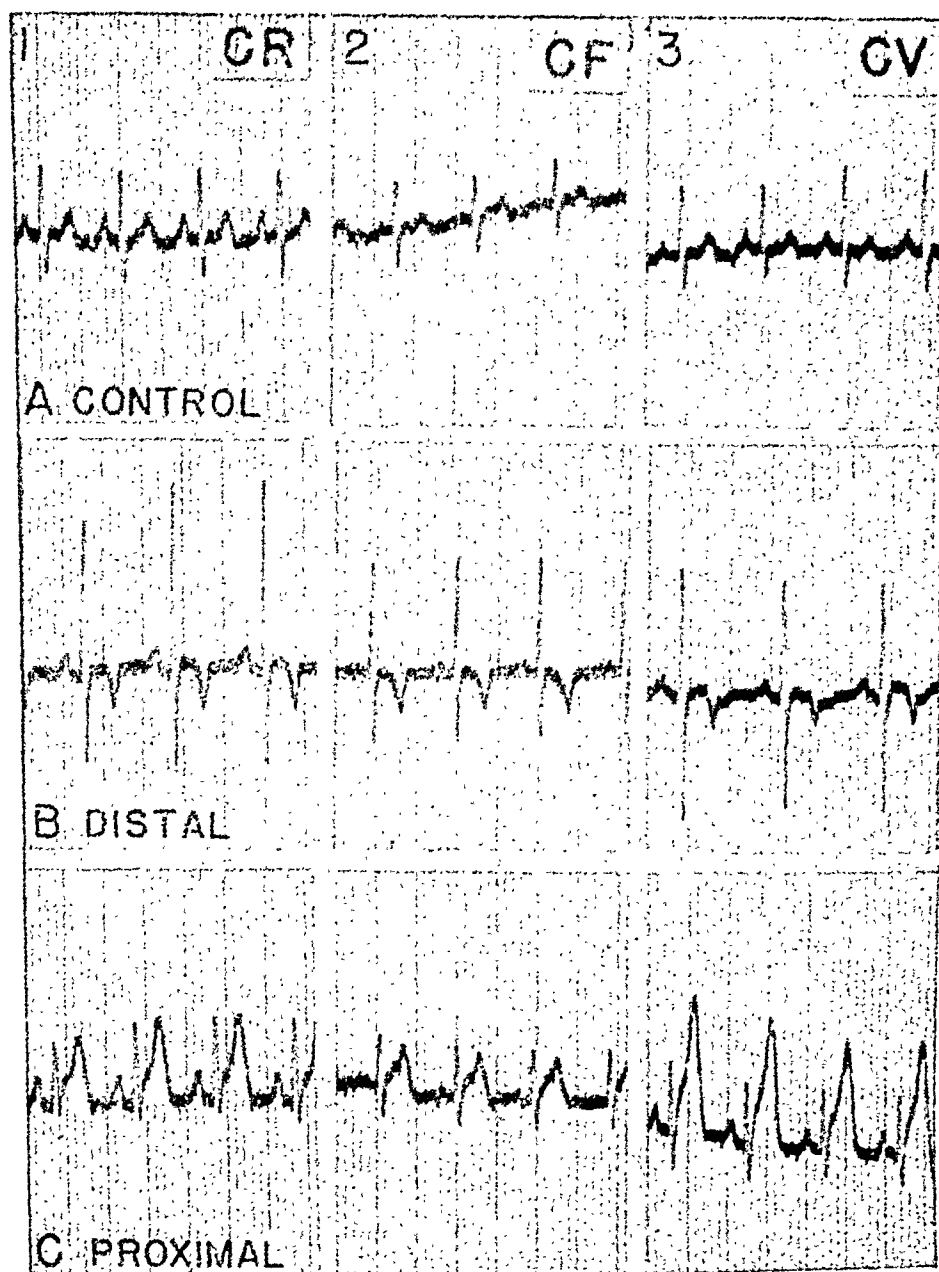


Fig. 4. SAME EXPERIMENT as fig. 1. Left apical precordial lead. A. Control. 1, CR; 2, CF; 3, CV. B. Warming posterior base (distal zone). T-wave becomes sharply inverted. C. Warming left apex (proximal zone). T sharply upright.

culminating in inversion. Similar treatment of intermediate areas was without influence on the T-wave. Cooling the proximal zones caused inversion of the T-wave while cooling the distal zone caused elevation of the T-wave (figs. 1, 4).

The reversible damage caused by application of 0.10 M KCl solution to the proximal area under the chest electrode always evoked an upward displacement of the RS-T segment, while damage to distal areas was always followed by a depression of the RS-T segment. Intermediate silent areas again separated distal and proximal zones (figs. 2, 5).

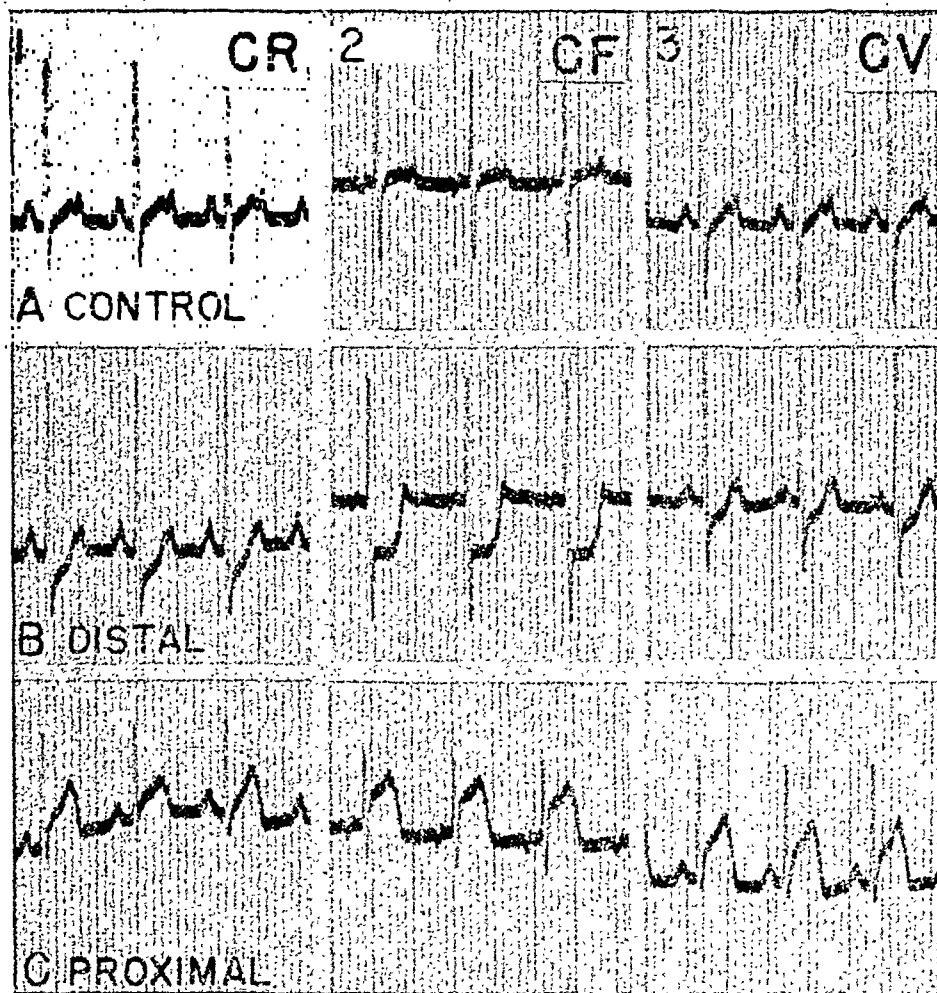


Fig. 5. Dog, same as fig. 1. Left apical lead. A. Control. 1, CR; 2, CF; 3, CV. B. Isotonic KCl applied to posterior base (distal zone). C. Isotonic KCl applied to left apex (proximal zone). S-T segment elevated in each lead.

Extrasystoles elicited from proximal areas invariably exhibited only a simple QS configuration, while those arising in distal areas showed only an R-wave (figs. 3, 6). Intermediate zones gave rise to QRS patterns in which R became more prominent as the point of stimulation approached the distal zone, while S was augmented as the proximal zone was approached.

With each position of the chest electrode, the proximal zone always included at least the apex of the ventricle corresponding to the side of the chest lead selected, while the distal zone always included some portions of the base of the heart. In other words, the chest leads always included an element of base-apex interference.

In general, the complexes were of greater amplitude and the extent of the proximal zone was less in the CR and CF leads than with CV leads. From the standpoint of sharpness of delimitation of the proximal area, therefore, leads CR and CF were

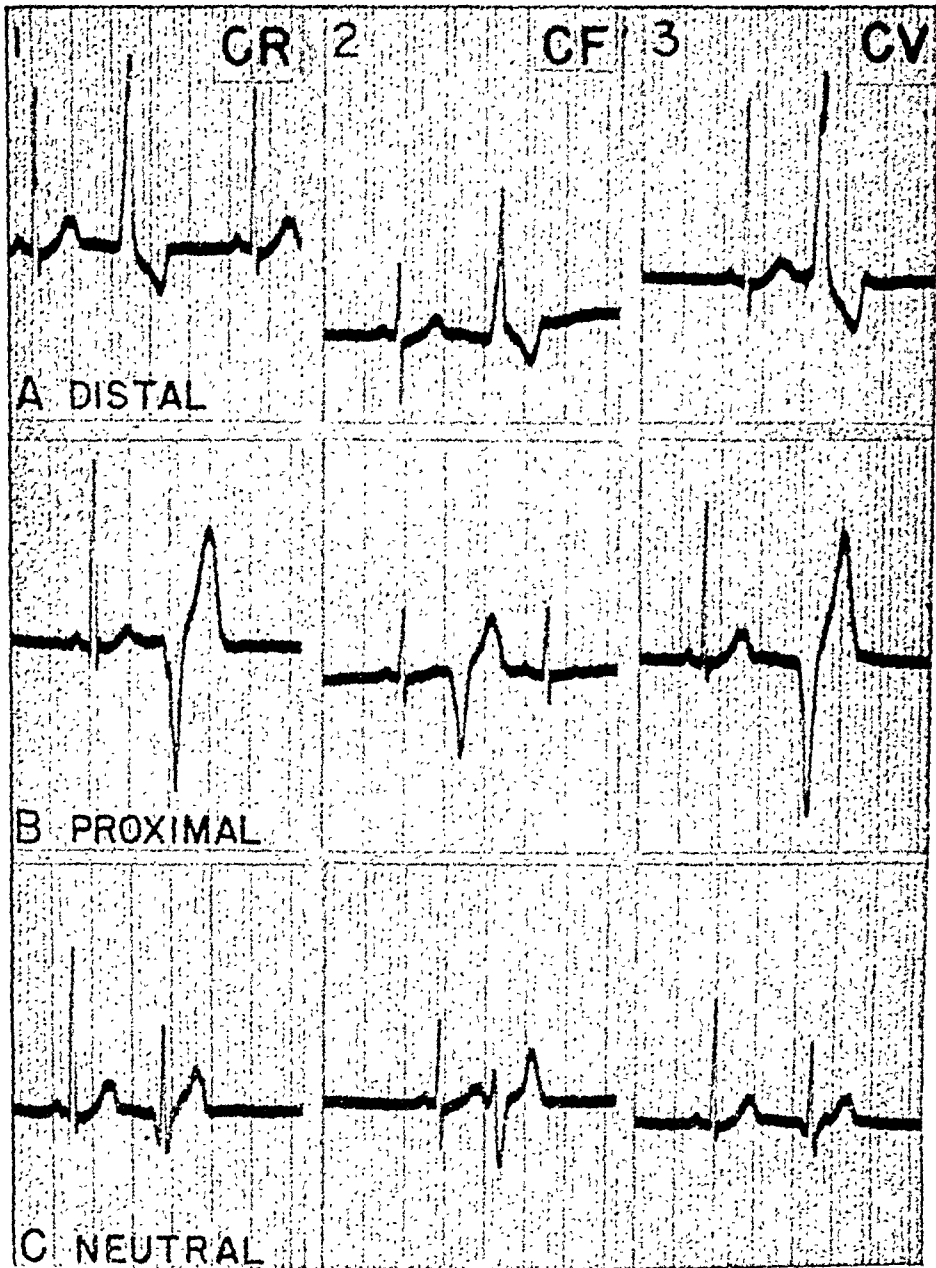
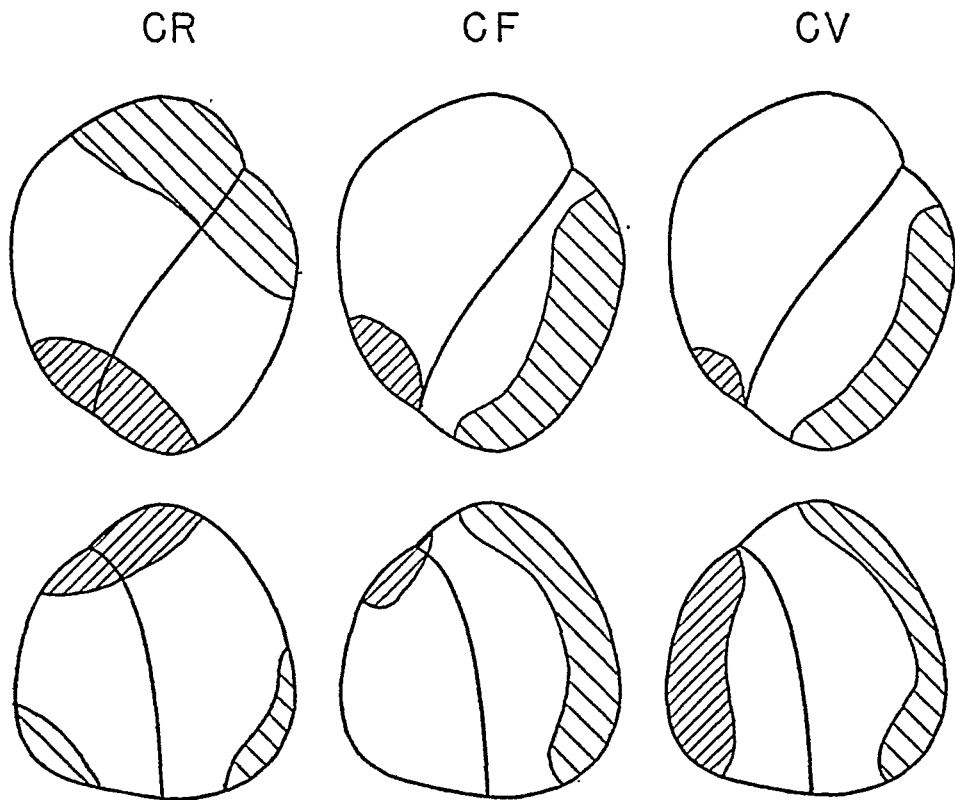


Fig. 6. Dog, August 31, 1944. Left apical lead. A. Extrasystoles by threshold stimulation of a point in the distal zone. 1, CR; 2, CF; 3, CV. Initial movement of the beam upward. B. Extrasystoles elicited by threshold stimulations of a point in the proximal zone. Initial beam movement downward. C. Extrasystoles elicited by threshold stimulation of a point in the neutral zone. Multiphasic QRS.

slightly superior to CV. In all leads studied the proximal and distal zones as mapped out by the extrasystole method could be defined more exactly than by the method of warming and cooling or local surface damage. Figures 7 and 8 summarize the data obtained in the various experiments for each lead.



[Fig. 7. SUMMARY of the proximal and distal zones of the dog heart when the lead is taken with the exploring electrode placed over the right apex; CR, CF, and CV leads. Above, ventral surface of the heart, apex down; below, dorsal surface of the heart, apex up. Fine shading, proximal zones; coarse shading, distal zones.

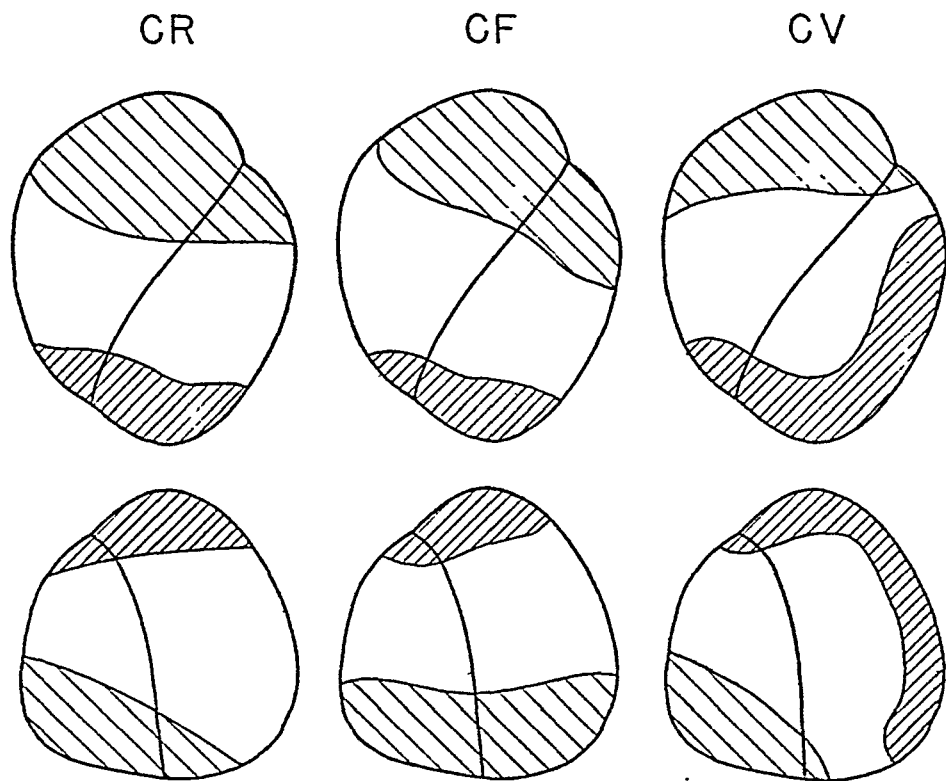


Fig. 8. SUMMARY of proximal and distal zones of the dog heart when the lead is taken with exploring electrode over the left apex. Fine shading, proximal zones; coarse shading, distal zones.



## DISCUSSION

When these experiments are considered in the light of the failure to establish any differences in the contribution of epicardial and immediately subjacent endocardial surfaces of the myocardium to the electrocardiogram, the conclusion appears justified that each precordial electrocardiogram reflects the interplay of two forces arising from the excitation and recovery of specific proximal and distal zones of the heart with respect to the position of the chest electrode.

The only force that can produce a downward movement in the QRS portion of the electrocardiogram is derived from excitation of the myocardium lying beneath and in close proximity to the chest electrode (the proximal zone). The direction from which the wave of excitation approaches makes no difference whatever, and in particular it is a matter of indifference whether the endocardial or epicardial surface of the myocardium is the first to be excited. Only excitation in the proximal zone can evoke a downward movement in the electrocardiogram, and by no means is it possible to produce such a movement by initial activation of the distal zone. If the region underneath the chest electrode is the first of all parts of the heart to be fully excited, a Q-wave or QS complex is produced, while the prior activation of a distal area is responsible for the appearance of an R-wave. The downstrokes of R and of S are also the result of proximal excitation, while the upstrokes of Q, R, and S indicate distal excitation. Reference to figures 6 and 7 shows that the areas involved in the proximal as well as distal zones are large enough to encompass both early and late subdivisions, and these account for various combinations of Q, R and S.

The T-wave is also formed from the summation of the electrical effects of recovery in proximal and distal areas. An initial upward movement indicates beginning recovery in the proximal zone, and downward movement indicates beginning recovery in the distal region. An upright T-wave develops when the distal region recovers later than the proximal zone, while an inverted T-wave results when the proximal zone recovers later than the distal zone.

The depression or elevation of the RS-T segment that develops with the establishment of localized areas of injury fits into the same pattern. Surface injury in a proximal zone produces elevation of the S-T segment, while surface injury in the distal zone produces a depressed RS-T segment. Surface injury in the intermediate zone caused no deviation in the RS-T segment.

The chest leads differ from the standard leads only in that the areas involved in the interference are unequal in size and are differently located, tending at times to have base-apex localization. The apex of the ventricle underneath the chest electrode was always included in the proximal area, as was also the apex of the opposite ventricle in some instances. Less frequently the proximal zone extended in a narrow projection toward the base, either anteriorly or posteriorly. The proximal zone was also considerably smaller than the distal zone, although this feature was less marked when CV leads were employed. The distal zone in these apical leads always included portions of the base, especially the lateral portions as its most important elements, and only infrequently were projections noted toward the apex of the ventricle opposite that under the recording electrode.

It is of course to be remembered that these experiments were carried out on the heart of the dog, and the particular topography of distal and proximal zones cannot be applied exactly to the human heart. A chest lead, taken from any point, will have its own particular zones of interference, depending upon the position of the electrode as well as the size and orientation of the heart. There seems no reason to doubt that the subdivision of the heart into two major interfering zones separated by a neutral area exists for the precordial lead of man as it does in the dog. There is furthermore no reason to believe that the proximal zone is proportionately any less extensive in man than in the dog.

#### SUMMARY

1. The precordial electrocardiogram represents the interference of opposing electrical forces developing with the excitation and recovery of specific regions which are proximal and distal with respect to the position of chest electrode. An intermediate zone rather large in extent separates the proximal and distal zones of each lead. Potentials derived from the excitation of this zone fail to be reflected in the precordial electrocardiogram.

2. The downstrokes of Q, R, and S are caused by preponderance of excitation in proximal areas. The upstrokes of Q, R, and S are caused by preponderance of excitation in distal areas.

3. The T-wave is similarly derived from differences in the time and rate of recovery from excitation in distal and proximal zones. An upright T-wave indicates beginning of repolarization in the proximal zone in advance of the distal region, while an inverted T indicates that regions in the distal zone begin to repolarize in advance of the proximal zone. The S-T segment is elevated when injury is present in the proximal zone, and depressed when injury occurs in the distal area.

4. Injury to the large intermediate zone cannot be detected in the precordial electrocardiogram. The proximal and distal zones for three different leads (CR, CF and CV) were determined in the dog when the chest electrode was placed external to the left and right apex.

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# DETERMINATION OF CIRCULATING RED BLOOD CELL VOLUME WITH RADIOACTIVE PHOSPHORUS<sup>1</sup>

ROBERT T. NIESET, BLANCHE PORTER, W. V. TRAUTMAN, JR., RALPH M. BELL, WILLIAM PARSON, CHAMP LYONS AND H. S. MAYERSON

*From the Laboratory of Biophysics, Departments of Medicine, Surgery and Physiology, Tulane University School of Medicine, and the Alton Ochsner Medical Foundation*

NEW ORLEANS, LOUISIANA

**D**IRECT measurement of total circulating red blood cell volume has been accomplished most accurately in man by an isotope dilution technic using red blood cells labelled with radioactive iron (1, 2). The method requires an available and previously prepared donor with labelled red cells and considerable technical skill in the handling of blood samples submitted for analysis. It is not a method that can be adapted easily to wide clinical exploitation.

This report has two purposes: first, to present a method for measuring the total circulating red blood cell volume in man by an isotope dilution technic using radioactive phosphorus; and second, to discuss sources of error in the proposed method.

The method is a modification of that proposed and used by Hahn and Hevesey (3), Anderson (4), and Brown *et al.* (5). It has many advantages, chiefly those of simplicity in the labelling of red blood cells and in the preparation of samples for counting. In all instances the blood of the subject to be studied has been used for labelling.

## METHOD

*Labelling of Red Blood Cells.* Ten (10) ml. of blood was withdrawn under aseptic precautions from the subject into a sterile heparinized syringe and transferred to a sterile centrifuge tube containing 50 microcuries of P-32 as phosphoric acid ( $H_3PO_4$ ) in 0.1 to 1 ml. of autoclaved solution. This mixture was incubated at 37°C., in a water bath, for 2 hours with mild manual agitation every 20 minutes (approximately 40% of the P-32 was taken up by red blood corpuscles during this period)<sup>2</sup>.

The mixture was then centrifuged and the supernatant discarded. The cells were resuspended in 8 to 10 ml. saline, washed by gentle agitation, and the mixture was centrifuged and the supernatant discarded. The cells were finally resuspended in saline to a total volume of 10 ml. and thoroughly mixed. This was the final suspension of labelled red blood cells. It was used in part for injection into the sub-

Received for publication September 27, 1948.

<sup>1</sup> Publication No. 8 from researches accomplished under a grant from the office of the Surgeon General, United States Army. A preliminary account of this method was presented before the meetings of the American Physiological Society on March 19, 1948, and was published in *Fed. Proceedings* 7: 85, 1948.

<sup>2</sup> We have also used the procedure of Anderson (4) of bubbling 5%  $CO_2$  and 95%  $O_2$  through the blood sample. This increases the rate of uptake of P-32 by the cells and shortens the incubation period considerably. The results given in this and the subsequent papers have, however, been obtained by the procedure described above.

ject of study and in part to obtain a count of the radioactivity of the labelled red blood cells.

*Injection of Labelled Red Blood Cells and Sampling After Injection.* An exactly measured quantity, usually 5 ml., of the final suspension of labelled red blood cells was taken up in a calibrated syringe. This was injected directly into an antecubital vein, care being taken to ensure complete delivery by aspiration and injection of blood to wash the syringe at least twice. After a lapse of 10 minutes, 4 to 6 ml. of blood were withdrawn from the opposite antecubital vein without a tourniquet into a heparinized syringe and transferred to a glass container.

*Handling of Blood Samples.* Two sources of blood were utilized: a) From the final suspension of labelled red blood cells, as prepared for intravenous injection, 1 ml. was added to a volumetric 50-ml. flask and made up to a volume of 50 ml. with tap water. Duplicate dilutions were made. The cells were laked during this process. One ml. of each of these laked 1:50 dilutions of the final suspension of labelled cells was added to each of 2 sample dishes for counting. b) From the heparinized whole blood withdrawn from the subject after mixing of the injected labelled cells, two determinations were prepared: 1) one ml. was added to each of two sample dishes, 2) a Wintrobe hematocrit tube was filled and centrifuged at 3000 r.p.m. (checked by tachometer) for 30 minutes. The radial distance from the axis of the centrifuge to the bottom of the tube was 20 cm. The observed red cell concentration was reduced by 8.5 per cent to correct for the quantity of plasma retained among the cells (6).

*Assay of Radioactivity of Samples.* a) *Preparation of sample dishes.* The sample dishes were prepared from  $\frac{1}{4}$ -ounce ointment tins (bottoms only). Filter paper discs were prepared to fit exactly into the tin. Rubber cement was spread in a thin layer on the tin and on one surface of the filter paper and allowed to dry for two to three minutes. The filter paper disc was cemented into the tin and allowed to dry at room temperature.

b) *Drying of samples.* The blood samples to be counted were allowed to run onto the filter paper. This assured even distribution. The samples were then dried without boiling, which is important. Drying is done most expeditiously in an oven at 60° to 80°C.

c) *Counting of samples.* The counter tube was arranged in a brass box with a sample-holder that fit exactly the ointment tin and ensured a uniform geometry of sample and counter. Variations of surface geometry were minimized by even distribution of the added blood, but duplicate samples reduced the error from this source to a  $\pm 2$  per cent. The counting was done with a thin glass walled, cylindrical beta counter.

*Calculations.* The counts per second of duplicate samples were averaged. Counts per second of diluted samples were recalculated to counts/sec/ml. of injected suspension. It was assumed that the counts per second of whole blood, as determined, represented the activity of the previously injected cells. The quantity of cells actually present in 1 ml. of the whole blood sample was determined as described above. The counts per second of a known quantity of cells were thus obtained. From this value, the counts per second of 1 ml. of cells was calculated.

The total circulating volume of red blood cells was calculated from the following formula:

$$\text{mls. of red blood cells} = \frac{\text{Total counts/sec. injected}}{\text{Counts/sec./ml. of cells withdrawn}}$$

#### SOURCES OF ERROR

During the developmental experience with this method, a number of controversial issues and apparent sources of error were visualized. Certain of these problems are considered below:

*Loss of P-32 from Labelled Cells.* The most likely source of serious error in the method is the possible loss of P-32 from the red cells to plasma and extravascular compartments, which would appear as excess dilution and give values for red cell volume that would be too high. Both *in vitro* and *in vivo* studies of such loss have been made.

Labelled cells have been repeatedly washed with both normal saline and unlabelled plasma, and the increase in activity of the washing observed. This increase is variable, but averages about 10 per cent of the total initial activity of the centrifuged cells. Most of the increase occurs within the first 10 minutes of the first washing. In subsequent washings only a small increase, exponential with time and dependent upon the volume of solution is observed. In large dilutions (1 to 1000 for red cells to saline) this amounts to less than 3 per cent of the activity of the cells.

There is reason to believe (6) that some plasma remains with the cells as a contaminant after the separatory centrifugation, and amounts to 8 or 9 per cent of the red cell volume. Since the P-32 content of the plasma from which the labelled cells was separated varies from 80 to 150 per cent of the content of the cells, an activity of 6 to 12 per cent of the total activity of centrifuged cells plus contaminant would be expected to appear in any washing solution which removed or diluted the contaminating plasma. This amount of activity is not truly present in red cells at any time before injection into a subject and is largely, but not completely, removed by the single washing employed in routine applications of the method. All of the true loss of activity from cells to solution, corresponding to the second observation above, and the activity of the residual labelled plasma carried over after the single washing is regarded as belonging to the cells of the suspension prepared for injection.<sup>1</sup>

The justification for this assumption appears in the results of two types of *in vivo* experiments. The first type of experiment was made possible through the use of large intravenous doses of P-32 for therapy. Here the loss of P-32 from the blood stream to the extravascular compartments and from plasma to red cells could be followed conveniently.

The results are represented in figure 1. The rate of loss from whole blood is shown to be approximately one-half the rate of loss from plasma. The difference between the loss from plasma and the loss from whole blood represents the transfer of active material from the plasma to the initially unlabelled red cells. Therefore, only half the P-32 in the plasma is assumed to leave the plasma to go into the extravascular compartments. The other half of it goes into red cells. If, therefore, an injected sample of labelled red cells either carries some contaminated plasma

along—or loses P-32 to the plasma after injection—only one half of it will be lost to extravascular compartments, the other half would return to more red cells. The 'loss' observed in previous experiments amounted to a maximum of 10 per cent; half of that we now concede lost to extravascular compartments; we are left with a 5 per cent loss which agrees well with the loss observed in successive samples as described below. Reeve and Veall (7) report a mean loss of about 6 per cent during the first hour after injection, but on occasion the loss appears to be negligible.

In the second type of experiment, blood samples are taken at intervals after the injection of labelled cells and the activities compared. Over long ranges of time, from one hour to 48 hours, the disappearance of the labelled material is remarkably

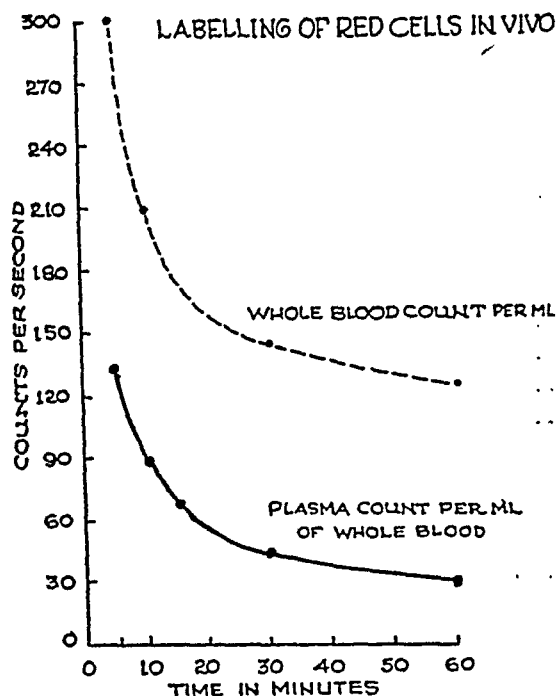


Fig. 1

constant from one subject to another, the average rate of loss being such that after the elapse of 24 hours,  $47 \pm 2$  per cent of the activity remains in the red cells. Extrapolation from the long-range (1 to 24 hours) disappearance curves gives a loss over the first 15 minutes of 2 per cent. The curve satisfies the relation,  $\log A_t - \log A_o = kt$  where  $A_t$  and  $A_o$  are the activities at time  $t$  and  $o$  respectively,  $k$  is a constant, and  $t$  is elapsed time. This indicates that the time loss of activity is small and constant.

However, when observations are confined to intervals of 5, 10, 15 and 30 minutes, no such smooth curve is observed and the apparent loss is much greater and variable as shown by the data in table 1. Even here, the average loss is not greater than 5 per cent and the extreme cases are included in this small selection of the data.

These variations in apparent active phosphorus concentration in the first 30 minutes after injection as compared with the smooth disappearance curve that is obtained over longer intervals suggests that other factors than loss of P-32 are at

work immediately after injection of the labelled cells. Nevertheless, the data may be taken as indicating an average loss of 5 per cent of the injected label over the time required for the volume determination.

*Errors of Self-absorption and Geometry in Counting.* Errors of self-absorption and geometry in counting were studied by making duplicate determinations of samples made up to different dilutions ranging from 1:1 to 1:1000. The standard error attributable to such sources was less than 2 per cent. Counts were made to an accuracy better than 1 per cent.

TABLE 1. PERCENTAGE OF ORIGINAL ACTIVITY (OF FIRST SAMPLE DRAWN) REMAINING AFTER VARIOUS INTERVALS

EXP. NO.	MINUTES AFTER INJECTION				
	5	10	15	20	30
1		100		102	
2	100		101	102	
3		100	97		
4	100	96	89		
5		100	99		
6	100	97			
7	100	98	97		
8	100	98		95	
9	100	86		77	
10		100		96	92
11		100		102	

TABLE 2. RESULTS OF DUPLICATE DETERMINATIONS OF RED CELL VOLUME

DISCREPANCY	NO. OF CASES
0- 2%.....	10
2- 5%.....	8
5-10%.....	1

*Duplicate Determinations to Check Overall Consistency.* A summary of the discrepancies observed between maximum and minimum values obtained in 19 sets of duplicate determinations of red cell volume is presented in table 2.

#### OBSERVATIONS

Measurements of total circulating red blood cell volume were made in a large number of healthy individuals and patients suffering from a variety of clinical disabilities. Some of these results are presented in the papers which follow. The results are in close agreement with similar measurements made by us and by others with different technics.

Clinical experience with the P-32 method has demonstrated its utility, particularly under circumstances where repeated determinations are desired. The activity of the tagged cells is  $\frac{1}{10}$  of the safe tolerance dose and 75 per cent excretion occurs

in 48 hours. For repeated determinations, therefore, only a small correction need be made for the residual activity of the patient's blood. This may be determined from the blood sample withdrawn for labelling.

#### SUMMARY

A method for the direct measurement of total circulating red blood cell volume by an isotope dilution technic using radioactive phosphorus has been presented. The red cells from the subject of study are utilized for labelling. Rapid uptake and slow release of radioactive phosphorus by exposed red cells facilitates wide experimental application. Ease of counting and the opportunity for repetitive measurement are other advantages.

The assistance of Dr. Robert Hutcheson and Miss Shirley Lis Robertson is acknowledged gratefully.

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# COMPARISON OF RESULTS OF MEASUREMENT OF RED BLOOD CELL VOLUME BY DIRECT AND INDIRECT TECHNIQUES<sup>1</sup>

H. S. MAYERSON, CHAMP LYONS, WILLIAM PARSON, ROBERT T. NIESET  
AND W. V. TRAUTMAN, JR.

*From the Departments of Physiology, Surgery and Medicine, and the Laboratory of Biophysics,  
Tulane University School of Medicine, and the Allon Ochsner Medical Foundation*

NEW ORLEANS, LOUISIANA

CONSIDERABLE controversy has arisen over discrepancies in the volume of circulating red blood cells in accordance with variations in the circumstances or methods of measurement (1-4). In general, the total circulating red blood cell mass is smaller when measured directly (radioactive iron technic, carbon monoxide method) than when calculated from the plasma-dye-hematocrit observations (T-1824 technic). We have made certain observations upon this discrepancy as a consequence of concomitant measurement of the red cell mass with the P-32 technic described in the preceding paper and of plasma volume with the T-1824 method as described by Gregerson (5).

Eighty sets of determinations were made upon 10 'normal' individuals and 35 patients hospitalized for various reasons. Determinations were made under basal conditions. Individuals in usual health omitted breakfast and rested in the laboratory for one-half hour before the test was performed. Hospitalized patients were allowed to remain quietly in bed without breakfast. The standard procedure was to withdraw 10 cc. of blood from an antecubital vein without stasis and to inject through the same needle 5 cc. of red cells tagged with P-32 (as described in the preceding paper). The syringe was rinsed twice with blood and 5 cc. of T-1824 solution was injected and the syringe rinsed twice again. After 10 minutes, 15 cc. of blood was withdrawn from the opposite antecubital vein and the sample divided for the determination of the red cell and plasma volumes.

In assessing the validity of the red cell volume as estimated from the plasma-dye-hematocrit values, two points are crucial. 1) The first relates to the accuracy of the *in vitro* determination of the hematocrit in peripheral venous blood. The usual value given for the peripheral venous hematocrit ignores plasma trapped between sedimented red blood cells in spite of the recognized existence of this source of error. Reluctance to correct for this error stems from uncertainty as to the correction factor to be applied. Chapin and Ross (6) have determined this factor as 8.5 per cent. Using similar methods, we have corroborated this value as applicable to the hematocrit determined by centrifuging in Wintrobe tubes at 3000 r.p.m. for 30 minutes. The distance from the axis of the centrifuge to the bottom of the tube is 20.0 cm. Correction on this basis has become standard practice in our laboratory.

2) The second point relates to the identity of the *in vitro* measurement of the

Received for publication September 27, 1948.

<sup>1</sup> Publication No. 9 from researches accomplished under a grant from the Office of the Surgeon General, United States Army.

peripheral venous hematocrit with the ratio of cells to plasma in the entire vascular bed, the 'body hematocrit'. Pertinent data on this issue can be obtained only by concomitant measurement of peripheral venous hematocrit, direct measurement of plasma volume and direct measurement of total red blood cell mass.

### RESULTS

Typical results obtained by simultaneously measuring the blood volumes of healthy and sick individuals with P-32 and T-1824 are given in table 1. The hematocrit values used in all of the calculations are the observed hematocrits corrected by the factor 0.915.

Comparison of the red cell volumes as obtained by the plasma-dye-hematocrit and the P-32 methods show a ratio of 0.96 for the entire series of 26 cases. All of the 6 cases in the normal group and ten of the 12 cases in the male hospitalized group have values which agree to within 10 per cent. Eleven of these 18 cases agree to within 6 per cent. There is a marked discrepancy in only one case (no. 16), in which a biliary fistula was present. The group of female patients show a somewhat greater range than do the other two groups, and the values obtained by the P-32 method are generally lower than those calculated from the plasma-dye method. All of the values, however, agree to within 15 per cent with the exception of case 24, a gravely ill patient, where the discrepancy is large (23%).

Ross *et al.* (7) have suggested the use of a second correction factor of 15 per cent to compensate for the error introduced by the unequal distribution of erythrocytes and plasma throughout the vascular system. They claim that the cell volumes of normal human subjects determined in this fashion compare very closely with the cell volume determined in the same subjects with the use of radioactive tagged cells. In table 1, we have applied this correction to our data ( $RVD \times 0.85$ ) and compared the values so obtained with those obtained by use of the P-32 method  $\left(\frac{RVP}{RVD \times 0.85}\right)$ .

Except for 6 of the female patients who showed low P-32 values as compared to the T-1824 values, the correction introduces a discrepancy approximately equivalent to the correction factor. The reason for the difference in these female patients is not obvious. As a group they were more seriously ill than the male group. On the other hand, several of the male patients (cases 12 and 14) were also in critical condition at the time they were studied but showed good agreement in their P-32 and T-1824 values. The number of cases is obviously too small for critical evaluation.

Of particular interest is the remarkably close agreement of the whole blood volumes as calculated from the plasma-dye-hematocrit values (TVD) and the sum of the plasma and the red cell volumes as determined directly by the T-1824 and P-32 methods (TBV). The latter value for whole blood volume is obviously the most accurate estimation since each component is measured directly and independently. Twenty-four of the 26 sets of values agree to within 5 per cent of each other. The two cases in which larger errors were found are cases 16 and 24 which showed large discrepancies in their red cell and plasma volumes. In spite of these obviously aberrant values, the ratio for the entire group is 0.99. Comparison of the whole blood volumes as calculated from the P-32 data and the hematocrit values yields

TABLE 1

CASE	SEX	WT., KG.	DIAGNOSIS	PLASMA VOLUME ML.		RED CELL VOLUME, ML.				TOTAL BLOOD VOLUME ML.					HCT P.C.		
				PVD	PVP	RVP	RVD	RVP RVD × 0.85	RVD × 0.85	TBV	TVD	TVP	TBV TVD	TBV TVP	PHC	BH	BH PHC
1	♂	82.0	Normal	3580	3610	2937	2900	1.01	2460	6517	6480	6547	1.01	1.00	45.1	44.8	0.99
2	♂	60.9	"	2780	2655	2065	2170	0.95	1750	4845	4950	4720	0.98	1.02	43.9	42.7	0.97
3	♂	61.9	"	2970	2840	1760	1830	0.96	1556	4730	4800	4600	0.98	1.03	38.2	37.2	0.97
4	♂	60.9	"	2740	2720	1976	1990	1.00	1690	4736	4730	4696	1.00	1.00	41.1	41.9	1.00
5	♀	53.2	"	2680	2480	1336	1190	0.90	1770	4016	4170	3716	0.96	1.08	35.6	33.3	0.93
6	♀	57.0	"	2320	2180	1304	1385	0.94	1180	3624	3705	3484	0.98	1.04	37.5	35.9	0.96
Av. normals				2845	2731	1596	1661	0.96	1631	4745	4866	4677	0.99	1.03	40.4	39.3	0.97
7	♂	84.5	T. B. arthritis	3470	3060	2280	2430	0.93	2060	5550	5700	5340	0.97	1.03	42.6	40.7	0.96
8	♂	59.9	Gastrectomy	3320	2840	1912	2210	0.86	1880	5232	5530	4752	0.95	1.10	40.3	36.5	0.91
9	♂	87.7	Duodenal ulcer	2980	2980	2471	2420	1.01	2060	5451	5400	5451	1.01	1.00	45.3	45.3	1.00
10	♂	94.1	Polycythemia	2430	2640	2780	2550	1.09	2170	5210	4080	5420	1.05	0.96	51.2	55.1	1.08
11	♂	63.2	Osteo. sarc.	2980	2980	2004	2000	1.00	1795	4984	4080	4684	1.00	1.00	40.3	40.2	1.00
12	♂	68.2	Gastric sarc.	3680	3660	1084	1090	1.00	930	4764	4770	4744	1.00	1.00	22.9	22.7	1.00
13	♂	72.7	Osteo. sarc.	2550	2650	1473	1310	1.04	1100	4023	3660	4123	1.01	0.99	35.7	36.7	1.03
14	♂	73.6	Retic. cell sarc.	2890	3158	842	766	1.09	650	3732	3746	4000	1.00	0.93	21.0	22.6	1.08
15	♂	68.2	Hepatitis	4100	4455	2145	1970	1.08	1670	6245	6070	6600	1.03	0.94	31.5	34.4	1.06
16	♂	57.0	Biliary fist.	2340	3080	1790	1350	1.32	1145	4130	3690	4870	1.12	0.84	36.6	43.8	1.19
17	♂	71.4	Duodenal ulcer	3960	3765	1475	1370	0.95	1335	5435	5530	5240	0.99	1.04	28.3	27.2	0.96
18	♂	60.9	Hepatitis	2500	2380	1766	1850	0.95	1570	4266	4350	4446	0.98	1.03	42.5	41.5	0.98
Av. male patients				3083	3137	1835	1861	1.03	1530	4910	4892	4973	1.01	0.99	36.6	37.2	1.02
19	♀	76.0	Hemolytic anemia	3760	3222	958	1120	0.86	950	4715	4880	4180	0.97	1.13	22.0	20.3	0.90
20	♀	57.7	Cholecystitis	2650	2620	1400	1410	1.00	1200	4050	4060	4020	1.00	1.00	34.8	34.6	1.00
21	♀	55.5	Rheumatic arth.	2590	2390	1220	1320	0.92	1120	3810	3910	3610	0.98	1.05	33.9	31.9	0.91
22	♀	57.7	Anemia	2340	2010	1116	1300	0.86	1100	3456	3640	3162	0.95	1.08	35.7	33.4	0.91
23	♀	67.6	Breast sarc.	2180	1880	1150	1310	0.87	1110	3330	3190	3930	0.96	1.09	37.6	31.6	0.82
24	♀	41.5	Carcinomatosis	2120	1627	912	1220	0.77	1035	3026	3340	2569	0.91	1.15	36.6	39.7	0.84
25	♀	66.6	Intest. fist.	3440	2930	1390	1640	0.85	1390	4830	5080	4320	0.95	1.10	31.2	28.8	0.89
26	♀	65.9	Breast sarc.	2125	2115	1295	1315	0.85	1290	3720	3910	3410	0.95	1.00	38.0	34.0	0.91
Av. female patients				2688	2349	1184	1354	0.87	1119	3868	4012	3338	0.96	1.08	34.0	30.9	0.91
Av. all subjects				2907	2801	1619	1701	0.96	1441	4555	4600	4451	0.99	1.03	36.7	35.8	0.98

PVD = plasma volume directly determined with T-1824. PVP = TVP - RVP.

RVP = red cell volume directly determined with P-32. RVD = TVD - PVD.

$$TBV = PVD + RVP. \quad TVD = PVD \times \frac{100}{1 - PHC} \quad TVP = RVP \times \frac{100}{PHC}$$

$$PHC = \text{observed hematocrit} \times 0.915. \quad BH = \frac{RVP}{TVV}$$

an average ratio of 1.03. Only *case 16* shows a discrepancy greater than 15 per cent and 23 cases show an agreement to within 10 per cent.

Table 2 illustrates the results of a series of four determinations in a patient suffering from a severe infectious hepatitis whose metabolism was being studied in connection with various dietary procedures. Again the agreement in values over the 17-day period is excellent and within the errors of the methods.

As previously indicated, a crucial point in the present study was the determination of the magnitude of the discrepancy introduced into the blood volume determination because of unequal distribution of red blood cells in the vascular system. This can be estimated by comparing the average body hematocrit with the observed

TABLE 2. PATIENT 15 WT. 68.2 KG.; SEVERE INFECTIOUS HEPATITIS

DATE 1948	PLASMA VOLUME, ML.		RED CELL VOLUME, ML.			TOTAL BLOOD VOLUME, ML.					HCT P.C.		
	PVD	PVP	RVP	RVD	RVP RVD	TBV	TBD	TBP	TBV TVD	TBV TVP	PHC	BH	BH PHC
April 27.....	3900	4180	1810	1700	1.07	5710	5600	5990	1.02	0.95	30.2	31.7	1.04
May 3.....	4560	4470	2240	2290	0.98	6800	6850	6710	0.99	1.01	33.4	33.0	0.99
May 8.....	3830	3720	1980	2040	0.97	5810	5870	5710	0.99	1.02	34.8	34.0	0.99
May 14.....	4100	4455	2145	1970	1.08	6245	6070	6600	1.03	0.94	32.5	34.4	1.06

TABLE 3. PATIENT D. COMPARISON OF SAMPLES FROM LARGE VESSELS

SOURCES OF BLOOD	TIME, MIN.	HEMATOCRIT P.C.		VOLUME T-32, ML.			VOLUME T-1824, ML.		
		Per.	Body	R. C.	Plasma	Total	R. C.	Plasma	Total
Antecubital V.....	0	30.2							
Left femoral A.....	10	30.2	30.0	1430	3300	4730	1470	3370	4850
Hepatic V.....	12	30.2	30.0	1670	3855	5525	1700	3900	5600
Superior V.C.....	30	30.2	29.5	1610	3740	5350	1660	3840	5500
Left subclavian.....	35	30.2	32.7	1610	3715	5325	1440	3300	4740

(corrected) venous hematocrit. Such a comparison appears in table 1 and indicates a remarkable close agreement between the two values. Thus the average ratio for the entire series is 0.98 and again in only two instances are the differences greater than 10 per cent. Half of the cases show differences of 4 per cent or less and can certainly be considered as being identical.

Corroborative observations upon the identity of the corrected venous hematocrit and the body hematocrit have been obtained from several sources. In experiments with venous catheterization and simultaneous P-32 and T-1824 measurements, the corrected hematocrit from femoral artery, hepatic vein, caval and auricular blood has been in essential agreement with the peripheral venous hematocrit and the body hematocrit. Typical findings are presented in table 3. Estimation of total protein and hemoglobin concentrations in a number of experiments further confirms the identity of samples taken from these sites. Observations in one patient at the

time of splenectomy for familial jaundice have permitted comparisons with blood from the splenic artery and vein as well. Blood samples drawn from one of the antecubital veins, the splenic artery and the splenic vein gave hematocrit readings of 34.5, 34.7 and 34.5 per cent respectively. Red blood cell volumes calculated from these same samples gave values of 2000, 2178 and 2000 cc. respectively.

#### DISCUSSION

It must be apparent that correction of the value obtained for the peripheral venous hematocrit is the cardinal feature of this attempt to resolve the discrepancies between values obtained for red blood cell mass by direct and plasma-dye-hematocrit methods of estimation. Regardless of variation in centrifugation technics, it is to be expected that some plasma will be trapped in the sedimented red cells. It has seemed more important to us to adopt a standard centrifugation technic which permits application of a constant correction factor.

The uncorrected hematocrit gives a falsely high percentage of red blood cells which may be directly reflected in sense and magnitude in the calculation of red cell volumes from plasma-dye-hematocrit values. Similarly, any method of whole blood volume estimation which depends upon the peripheral venous hematocrit and a single direct measurement of either cells or plasma is open to error from this source.

Mathematical analysis of the predictable error indicates that failure to correct the venous hematocrit when a value of 40 per cent is obtained in the plasma-dye-hematocrit method would estimate a red cell mass 14 per cent in excess. Calculation of the whole blood volume would be too large by 5.3 per cent. In measurements of red cell volume by radioactive iron, the venous hematocrit is not used. However, estimation of whole blood volume by radioactive iron demands reference to the venous hematocrit and yields a value too small by 8.5 per cent.

Failure to correct for the error of the hematocrit is unquestionably responsible for many of the discordant results reported in the literature. Thus, if one analyzes the first five cases of comparative values for the plasma-dye-hematocrit and radioactive iron measurements as given by Gibson and his co-workers (1) the dye method yields red cell volumes 15 per cent higher than the iron method. Inasmuch as there was no correction of the hematocrit a discrepancy of 14.8 per cent was predictable. The average difference in whole blood volumes was 16.7 per cent and the average hematocrit was 42.6. Furthermore, applications of the correction factor of 8.5 per cent to the average value of 42.44 per cent for the venous hematocrit in the 40 normal males yields a value of 38.89 per cent. The average body hematocrit was 38.3 per cent. Correction of the average value for 40 dogs yields a value of 41.8 per cent for the venous hematocrit. The average body hematocrit was 41.5 per cent. In the same manner, correction of the peripheral venous hematocrit values in the 28 cases given by Meneely *et al.* (2) yields an average of 35.96 per cent as compared to their average body hematocrit value of 34.9 per cent. Reeve (8) has also discussed this point in a recent review.

On the other hand, those investigators who have used corrected hematocrits

have reported reasonably good agreement between the values determined from the red cell and plasma volumes respectively. Thus Root, *et al.* (3), who used a factor of 0.96 to correct the hematocrit values, reported that in 13 of 14 measurements on resting and working men and in 23 of 29 animal experiments the blood volumes determined by the CO method agreed to within  $\pm 10$  per cent with those obtained by the plasma-dye-hematocrit method. Likewise, in all of their 14 human experiments and 26 of the 29 animal experiments, the ratio of body to peripheral venous hematocrit was between 0.90 and 1.08.

While the correction of the peripheral venous hematocrit serves to reconcile many of the discrepancies reported in the literature with respect to the plasma-dye-hematocrit and the various red cell volume methods, it may not be adequate to explain differences under conditions where there are shifts of plasma or blood from one area to another. Since there is no proof that any of the available methods measure absolute blood volume, variations in vasomotor tone and blood flow may significantly influence the hematocrit ratios. Trapping and loss of plasma from the active circulation might be expected to occur after transfusion, hemorrhage and shock which would make for discrepancies in the peripheral and body hematocrits which could not be reconciled by merely correcting the peripheral hematocrit.

The results of the present study do not disprove the claims that the cell to plasma ratio is less in the capillaries than in the large vessels. They merely indicate that the amount of blood present in the small vessels is not large enough for the unequal distribution of red cells in these vessels to affect greatly the estimation of the blood volume as calculated from the dye and hematocrit reading under the conditions which we have studied. Root *et al.* (3) arrived at similar conclusions from their comparison of the dye and the CO methods.

Routine correction for trapping in the small vessels would seem to be unnecessary and would introduce a definite error in the calculation of the blood volume. Our experience indicates that the plasma-dye method and the radioactive phosphorus methods measure circulating plasma and red cell volumes respectively with average discrepancies of less than 5 per cent and that whole blood volume can be calculated with the same accuracy from either determination if the corrected hematocrit value is used. The random distribution of the data suggests that when large discrepancies are present, they may be due to errors in technic rather than to a fundamental and systematic error.

#### SUMMARY

Concomitant measurements of red cell mass and plasma volume have been made with the P-32 technic and the T-1824 method respectively in 10 'normal' and 35 hospitalized individuals. A standard correction factor of 0.915 was used to correct the hematocrit values for trapped plasma. Total blood volumes were calculated from the red cell volume and hematocrit and from the plasma volume and hematocrit respectively. These values were compared with the total blood volume as calculated from the sum of the actually determined red cell and plasma volumes and showed satisfactory agreement. Comparison of the peripheral and body hematocrits

also showed good agreement. The data suggest that the whole blood volume can be measured with an average discrepancy of less than 5 per cent by the plasma-dye-hematocrit method providing the corrected hematocrit value is used.

We are grateful to Dr. Robert Hutcheson, Blanche Porter and Shirley Lis Robertson who assisted in the work. The catheterization experiments were done by Dr. W. D. Davis, Jr. to whom we are indebted.

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# EFFECT OF THE ADMINISTRATION OF ADRENALIN ON THE CIRCULATING RED CELL VOLUME<sup>1</sup>

WILLIAM PARSON, H. S. MAYERSON, CHAMP LYONS, BLANCHE PORTER  
AND W. V. TRAUTMAN, JR.

*From the Departments of Physiology, Surgery and Medicine, Tulane University School of Medicine, and the Alton Ochsner Medical Foundation*

NEW ORLEANS, LOUISIANA

THERE is now considerable evidence that in man there are no reserves of blood or blood cells which are subject to emergency mobilization (1-4). In spite of these observations, there has been considerable reluctance to abandon the earlier concept of splenic reserve of red blood cells as set forth by Barcroft in his original work on animals (5). Textbooks in physiology still discuss this emergency function of the spleen (6) and the concept is emphasized frequently in clinical teaching. As a result of increasing experience with blood volume technics, particularly with the tagged-red cell volume method described in the preceding paper (7), it became of interest to repeat certain earlier studies and collect simultaneous determinations of pulse rate, plasma volume, red cell mass, whole blood volume, plasma protein concentration, peripheral hematocrit, hemoglobin concentrations and body hematocrit after the subcutaneous injection of 1 mg. of adrenalin. It seemed especially pertinent to a final decision about the existence of reserves of red cells to compare the peripheral venous hematocrit with the body hematocrit before and after the adrenalin response. The term 'body hematocrit' is used to express the ratio of plasma volume to red cell mass as measured directly.

We have studied the changes induced by the subcutaneous injection of 1 mg. of adrenalin in 5 subjects. Three of these were healthy adults (2 males and 1 female) and 2 (1 male and 1 female) were hospitalized patients suffering from rheumatoid arthritis and hemolytic anemia respectively. One normal male, who expected an injection of adrenalin, was given an injection of 1 cc. of normal saline. All observations were made under basal conditions.

Simultaneous plasma and red cell volumes were determined with T-1824 (8) and P-32 (7) respectively and the total blood volume and body hematocrits calculated from these values. The peripheral (*in vitro*) hematocrit was determined by centrifuging blood samples for 30 minutes at 3000 r.p.m. in Wintrobe tubes. The distance from the axis of the centrifuge to the bottom of the tube was 20 cm. Hemoglobin was estimated as oxyhemoglobin and the falling drop method, checked by Kjehldahl determinations, was used for protein determinations. After control levels had been established, 1 mg. of adrenalin was injected subcutaneously and blood samples were obtained at approximately five-minute intervals for 30 minutes.

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Received for publication September 27, 1948.

<sup>1</sup> Publication No. 10 from researches accomplished under a grant from the Office of the Surgeon General, United States Army.



The results are given in figure 1. A satisfactory 'adrenalin response' was obtained in every case (including the patient who received the injection of saline) as

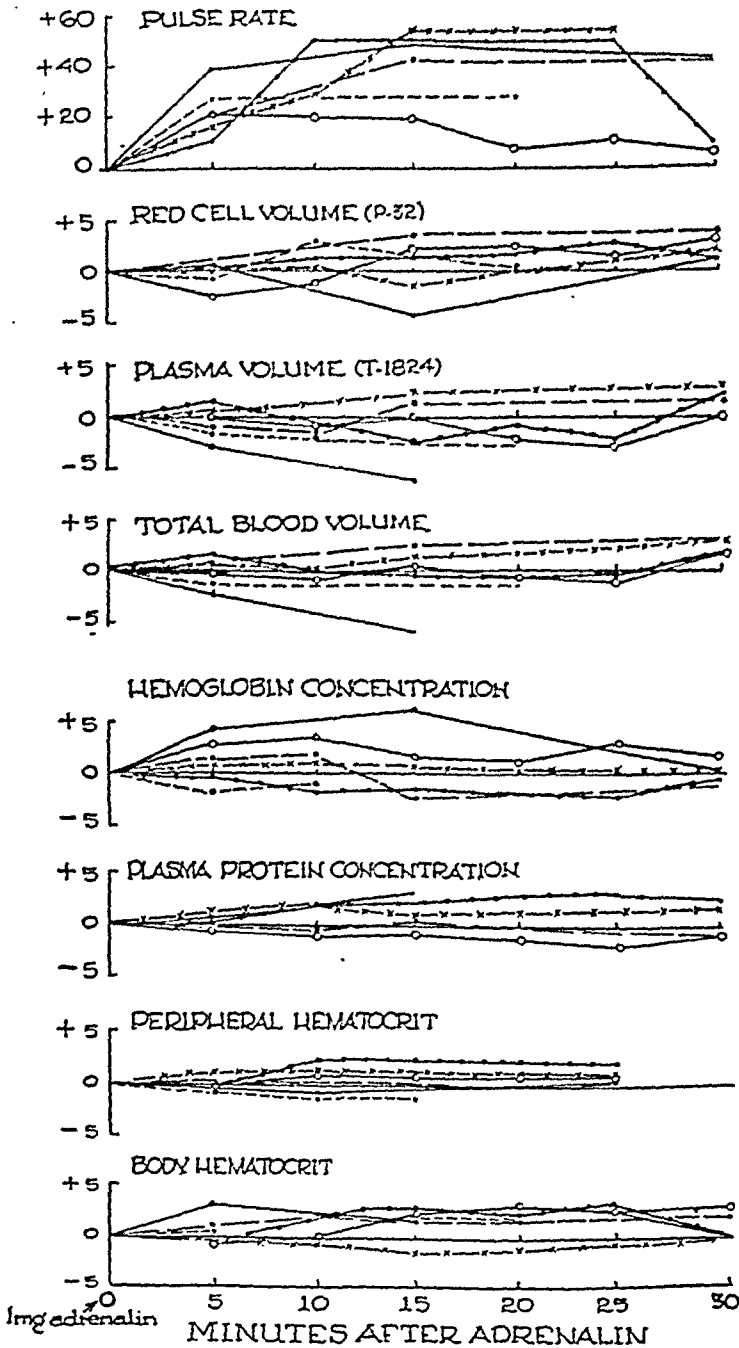


Fig. 1. EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF 1 mg. adrenalin. The values plotted are the percent changes from the average control (pre-injection) levels . . . . . = T.B.C. arthritis; ————— = hemolytic anemia; - - - - - = normal-1 mg. saline injected instead of adrenalin; -X-X-X- = normal male; - - - - - = normal male; -O- = normal female.

attested by the rise in pulse rate and the subjective reactions of the subjects. The red cell, plasma and total blood volumes and the body hematocrit showed minor

changes which are within the error of the methods. The protein concentration and peripheral hematocrit showed a tendency to rise, but the hemoglobin concentration showed no definite trend. None of the changes, except for those in the pulse rate, were significantly greater than the changes observed in these functions during the control period before the administration of adrenalin.

Our results thus confirm and extend those of previous workers who failed to find any evidences of mobilization of red blood cells after the injection of adrenalin. The relatively consistent finding of increased peripheral hematocrit and protein concentration has suggested that the injection of adrenalin results in hemoconcentration. Kaltreider *et al.* (2) measured the plasma volume after adrenalin with T-1824 and reported a slight decrease in plasma volume in 8 of their 10 subjects. These changes were less than 8 per cent in all except one case. Our results fail to provide evidence for hemoconcentration, since, as mentioned above, the changes in plasma volume are random and within the errors of the method.

Ross and Chapin (3) have suggested that the increase in peripheral hematocrit is due to a redistribution of circulating cells and plasma within the vascular system. Such redistribution should manifest itself as a difference in the peripheral and body hematocrit (9). If redistribution does occur, the magnitude is such that it cannot be demonstrated because of the relative insensitivity of the methods in our experiments.

#### SUMMARY

The subcutaneous injection of adrenalin in an amount sufficient to evoke a good clinical response does not result in any uniform or significant changes in the plasma or red cell volumes as directly measured by the T-1824 and P-32 technics. If sympathetic stimulation or adrenalin influence these functions, the effect must be very slight and of no real significance as an emergency response.

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# EFFECT OF STEROIDS ON THE BODY WEIGHT, TEMPORAL MUSCLE AND ORGANS OF THE GUINEA PIG<sup>1, 2</sup>

CHARLES D. KOCHAKIAN, JANE HARRISON HUMM<sup>3</sup> AND MARY N. BARTLETT

*From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

THE preliminary report by Papanicalaou and Falk (1), that testosterone propionate increases the muscle mass of the female and the castrated male guinea pig, provided direct evidence that the protein-anabolic activity of androgens (2) was not directed entirely to the regeneration and maintenance of the accessory sex organs and other internal organs. It seemed advisable, therefore, to confirm (3) the myotrophic property of this androgen and to determine whether there might be steroids with a greater myotrophic than androgenic effect comparable to the renotrophic-androgenic effect observed in castrated mice (4-6).

## METHODS

*Animals.* Immature, male, short-haired English albino guinea pigs were purchased from the Rockland farms. They were placed in individual metal screen cages (Norwich), fed *ad libitum* the Rockland guinea pig diet fortified with vitamin C and in addition a 12.5 mg. tablet of the vitamin was given by mouth each week. When the animals attained a body weight of approximately 250 gm., they were castrated in groups of 10 to 20 and treatment was begun 35 days later. At least one normal animal was included in the first five groups.

All of the steroids were studied for a 30-day period. In addition testosterone propionate as pellets was studied for 90 days, and for 14 days by injection at a dosage of 12.5 mg/day. Finally, one group of guinea pigs was castrated at approximately 600-gm. body weight, immediately implanted with testosterone pellets and compared with their normal controls 30 days later. Body weights were determined at approximately five-day intervals and on the day before and at autopsy.

*Steroids.*<sup>4</sup> The pellets of the various steroids were prepared as previously described (4). They weighed approximately 14 mg. each and were implanted subcutaneously by means of a trocar. The number of pellets implanted was determined on the basis of previous studies (3) and a series of preliminary experiments (7). The testosterone propionate<sup>4</sup> used for injections was provided in sesame oil at 25 mg/ml.

*Autopsy.* The guinea pigs were fasted 24 hours prior to autopsy when they were anesthetized by an intraperitoneal injection of dial-urethane<sup>4</sup> (usually 1.5 ml/kg. body wt.) and bled to death by cutting the blood vessels of the neck. The organs and temporal muscles were removed and weighed on a suitable Roller-Smith torsion balance.

A uniform portion of the ocular muscle was included with the temporal muscles because of its intimate attachment. The moisture content was determined by drying the muscle in an electric oven at 95 to 105°C. The non-protein nitrogen was determined on the trichloroacetic acid filtrate of fresh muscle. Total nitrogen was determined on aliquots of the acid hydrolysate of the dried muscles. The nitrogen determinations were carried out by the micro-Kjeldahl technic.

Received for publication July 19, 1948.

<sup>1</sup> This investigation was supported by the Josiah Macy Jr. Foundation.

<sup>2</sup> Parts of these data have been reported in the Josiah Macy Jr. Foundation Conferences on the Metabolic Aspects of Convalescence, 16th meeting, New York, 1947.

<sup>3</sup> Part of these data was taken from the M.S. thesis of Jane Harrison Humm.

<sup>4</sup> The steroids and dial-urethane were provided by Ciba Pharmaceutical Products, Inc.

## RESULTS

*Thirty-day Experiments*

*Body weight.* The castrated guinea pigs did not gain as much weight as the normal animals (table 1). Implantation of pellets of the various steroids restored the body weight toward normal, but when a maximum response was attained further increase in dose either had no further effect or was less effective.

TABLE 1. EFFECT OF STEROIDS ON BODY WEIGHT, KIDNEYS AND ADRENALS OF CASTRATED GUINEA PIGS<sup>1</sup>

	NO. OF G. PIGS	STEROID		BODY WEIGHT		KIDNEYS	ADRENALS
		Pellets	Absorbed	Initial	Change		
		No.	mg/30 days	gm.	gm., %	gm., %	gm., %
Castrate	15			460	(140)	(4.100)	(0.244)
Normals	6			507	47	-2	+13
Androstanol-17 $\alpha$ , one-3	5	1	1.9	467	41		
	6	3	5.0	464	38	10	-22
	5	6	9.1	449	30	2	-11
17-Methylandrostanol- 17 $\alpha$ , one-3	5	1	1.5	455	-13	-6	
	5	3	4.4	469	39	6	-14
Testosterone propionate	5	1	4.0	457	5		
	6	2	7.5	464	52	2	0
Testosterone	5	1	7.4	446	51	-1	7
	5	3	18.9	458	45	2	13
	5	5	32.2	448	35	16	
17-Methyltestosterone	5	1	6.8	478	36	5	3
	5	3	17.7	446	44	8	11
	5	5	28.3	451	39	6	
Androstanediol-3 $\alpha$ , 17 $\alpha$	5	3	5.8	455	19	-2	5
	4	12	14.5	488	43		
17-Methylandrostanediol- 3 $\alpha$ , 17 $\alpha$	5	1	1.9	466	-13	-9	
	5	6	9.5	454	33	2	-16

<sup>1</sup> Average values of the control guinea pigs are given in parentheses. The differences are from these values.

*Kidneys and adrenals.* There was no significant change in the size of these organs either as a result of castration or the implantation of the various steroids as pellets (table 1).

*Myotrophic effects.* Castration decreased the weight of the temporal muscles to less than one-third that of the normal animals. All of the steroids increased the weight of the temporal muscle of the castrated guinea pig, but in no instance was there a restoration to the normal size (fig. 1). Indeed the greatest increase was only about one-half that of the normal animals.

The myotrophic property was different for each steroid. Androstanol-17 $\alpha$ ,one-3 proved to be the most potent compound. Testosterone and testosterone propionate had the same effect and androstenediol-3 $\alpha$ ,17 $\alpha$  was very ineffective at a low dose level but on increasing the dose became very effective. The 17-methyl derivatives especially methyl testosterone were less effective than their parent steroids.

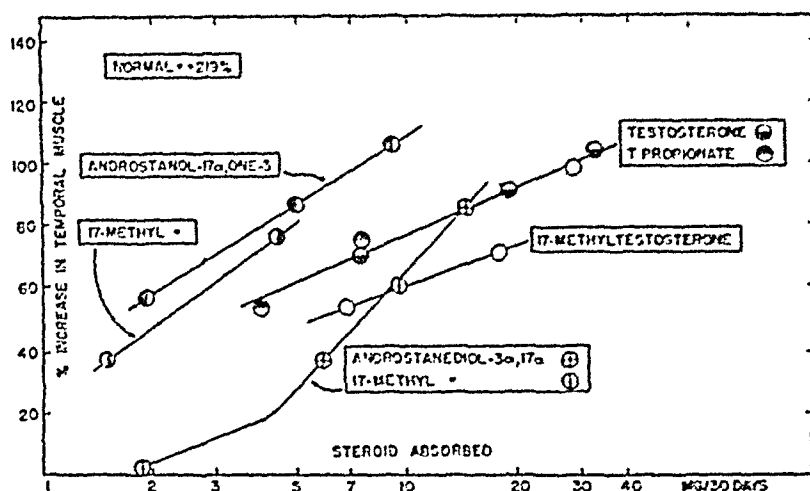


Fig. 1. Myotrophic property of steroids

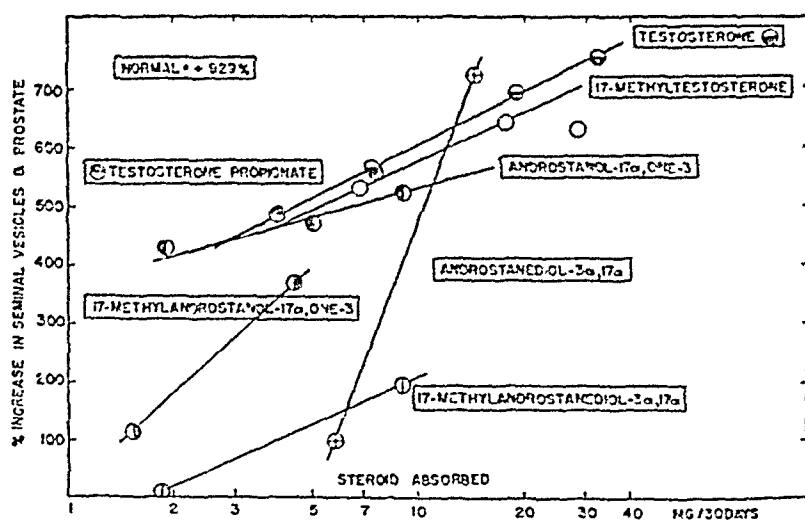


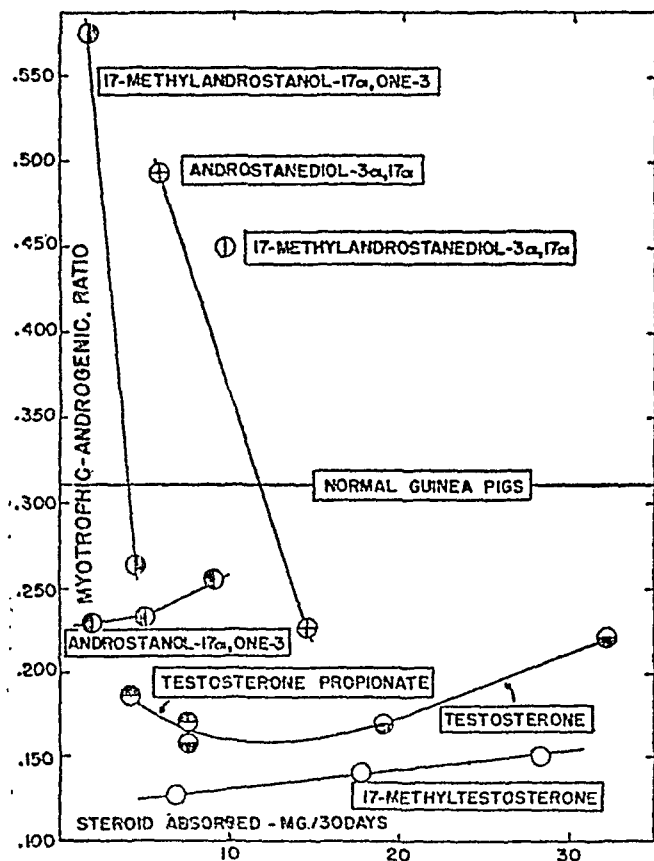
Fig. 2. Androgenic property of steroids

*Androgenic effects.* There was a definite stimulation of the seminal vesicles and prostates of the castrated guinea pigs by all of the steroids (fig. 2). At the higher dose levels they produced accessory sex organs only slightly smaller than those of the normal guinea pigs. The order of androgenic potency of the steroids was different from their myotrophic effects. Testosterone and testosterone propionate were the most potent. The 17-methylated compounds were again less effective than their parent steroids.

*Myotrophic: androgenic ratio.* The relative myotrophic-androgenic property

of the various steroids is illustrated by dividing the increase in temporal muscle mass by that of the seminal vesicles and prostates (fig. 3). The normal guinea pigs have a ratio of 0.311 and all of the steroids, except the diols and 17-methylandrostanol-17 $\alpha$ ,one-3, have lower ratios. As the dose of steroid exhibiting the high ratios is increased, there is a much greater increase in androgenic than myotrophic effect resulting in a rapid decrease of the ratio. The decrease, however, is not sufficient to lower the ratio below that of the other steroids at comparable dose levels. The steroids with the low ratios, however, progress towards the normal with further increase in dose.

Fig. 3. Effect of dose on the myotrophic-androgenic ratio.



*Ninety-day experiments.* The extension of the time of stimulation by testosterone propionate pellets to 90 days indicates that the seminal vesicles and prostates have been nearly returned to normal (fig. 4). Indeed the nature of the growth curve suggests that a further 30 days of treatment would have completely restored the size of these organs. The temporal muscles, on the other hand, are still very much smaller than those of the normal guinea pig and it would require an indefinite period of stimulation at the dose employed to accomplish complete restoration. The myotrophic and androgenic ratio was 0.323 for the normal guinea pigs and 0.167 for the androgen treated animals.

*Injection of a large dose of testosterone propionate.* The subcutaneous injection of 12.5 mg/day of testosterone propionate produced effects similar to those seen

by the subcutaneous implantation of pellets (table 2). This large dose of androgen was unable to restore the temporal muscle or the accessory sex organs to normal.

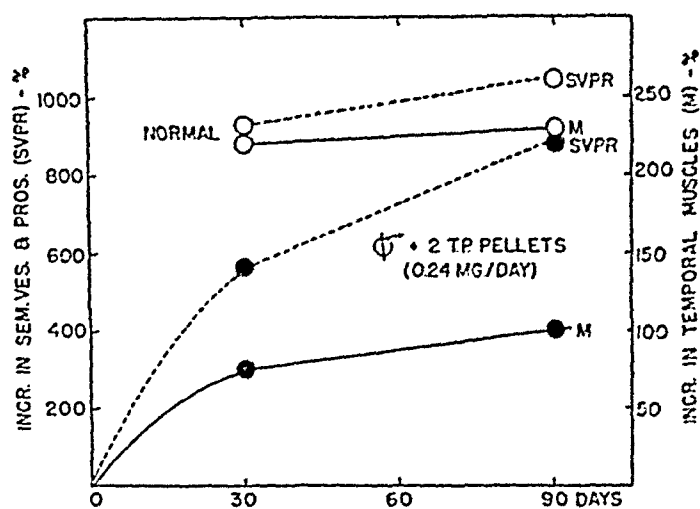


Fig. 4

TABLE 2. EFFECT OF INJECTION OF A LARGE DOSE OF TESTOSTERONE PROPIONATE (T.P.) ON CASTRATED MALE GUINEA PIGS

	NO. OF G. PIGS	BODY WT.		TEMPORAL MUSCLE (M)	SEM. VES. + PROS. (A)	M/A	KIDNEYS
		Initial	Change				
Control.....	5	575	gm., % (56)	gm., % (0.640)	gm., % (0.384)	mg/mg.	gm., % 4.280
T.P. 12.5 mg/day.....	5	579	+39	+50	+462	0.180	0

TABLE 3. MAINTENANCE OF THE TEMPORAL MUSCLE OF CASTRATED GUINEA PIGS BY TESTOSTERONE

	NO. OF PIGS	PELLETS	AB- SORBED  mg/30 days	BODY WEIGHT		TEMPORAL MUSCLE	SEM. VES. AND PROS.	KIDNEY
				Initial	Gain			
				gm.	gm., %	gm., %	gm., %	gm., %
Normals.....	4			602	(120)	(1.900)	(3.510)	(4.800)
Testosterone <sup>1</sup> .....	4	3	21.3	604	-7	0	+43	0
Testosterone.....	2	5	33.3	580	+50	-5	+74	+8

<sup>1</sup> Pellets implanted at time of castration.

Indeed the myotrophic and androgenic effects were equivalent to that observed after the absorption from a pellet of 0.1 mg/day for 30 days (table 1).

*Maintenance of temporal muscles and accessory sex organs.* Since it was so difficult in the preceding experiments to restore the size of the temporal muscle to normal size, it seemed important to determine whether these could be maintained

at the normal level. Therefore, a group of guinea pigs were castrated 35 days after the usual time and at the same time were implanted with pellets of testosterone. At autopsy 30 days later (table 3) the temporal muscles had been perfectly maintained at a dose of 0.7 mg/day. At the higher dose level of 1.1 mg/day there was no

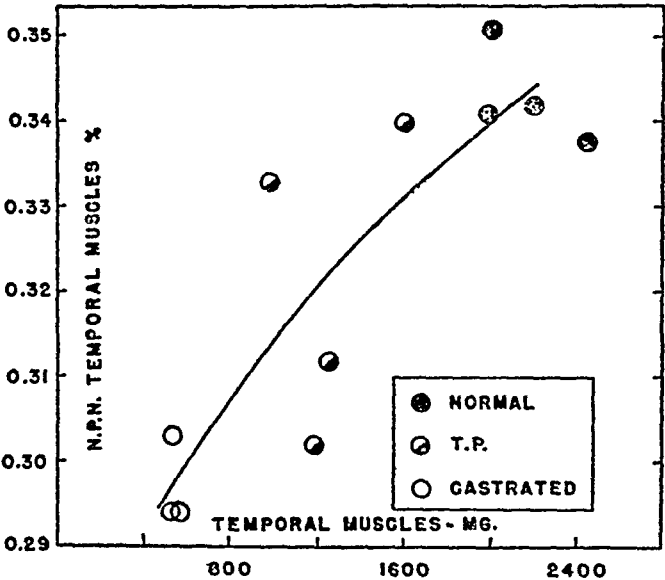
TABLE 4. COMPOSITION OF THE TEMPORAL MUSCLES OF GUINEA PIGS TREATED WITH VARIOUS ANDROGENS

	NO. OF G. PIGS	STEROID ABSORBED	TEMPORAL MUSCLE	WATER	PROTEIN (Nx6.25)	REST
		mg/30 days	mg.	%	%	%
Normal.....	6		1746	77.5	19.05	3.45
Control.....	6		547	76.1	17.95	5.95
Testosterone.....	5	7.4	931	76.5	18.95	4.55
Testosterone.....	5	18.9	1044	76.6	19.50	3.90
Testosterone propionate.....	6	7.5	958	76.3	18.85	4.85
17-Methyltestosterone.....	5	6.8	836	75.8	19.00	5.20
17-Methyltestosterone.....	5	17.7	935	76.3	19.00	4.70
Androstanol-17 $\alpha$ , one-3.....	6	5.0	1017	76.6	18.90	4.50
17-Methylandrostanol-17 $\alpha$ , one-3.....	5	4.4	963	76.2	18.45	5.35
Androstanediol-3 $\alpha$ , 17 $\alpha$ .....	5	5.8	757	76.2	18.27	5.53
17 Methylandrostanediol-3 $\alpha$ , 17 $\alpha$ .....	5	9.5	877	76.1	19.15	4.75

*T.P. injected at 12.5 mg/day for 14 days*

Control.....	4		640	75.7	18.20	6.10
Testosterone propionate.....	4		960	75.7	18.57	5.73

Fig. 5



greater stimulation. The accessory sex. organs, on the other hand, were greatly increased at the lower dose and further stimulated at the higher dose. The kidneys showed no significant changes. The increase in body weight was unaffected at the lower dose but definitely enhanced at the higher dose.



*Composition of the temporal muscles.* There is a slight decrease in both percentage of total water and total protein (nitrogen  $\times 6.25$ ) of the temporal muscles as a result of castration. The protein but not the water content is restored toward the normal level by the steroids (table 4).

In one series of experiments the nitrogen not precipitable by trichloroacetic acid (N.P.N.) was determined. Castration decreased the percentage of this mixture of nitrogen containing substances but testosterone propionate restored them towards normal (fig. 5).

#### DISCUSSION

It is noteworthy that as in the case of the renotrophic and androgenic effects in mice (4-6) none of the steroids was able to simulate the normal myotrophic and androgenic status of the normal guinea pig. It seems, therefore, that neither testosterone, in spite of its isolation from bulls' (8) and horses' (9) testes, nor any other of the steroids are 'the hormone' of the guinea pig or mouse testes. Therefore, if a single substance is primarily responsible for the development and maintenance of

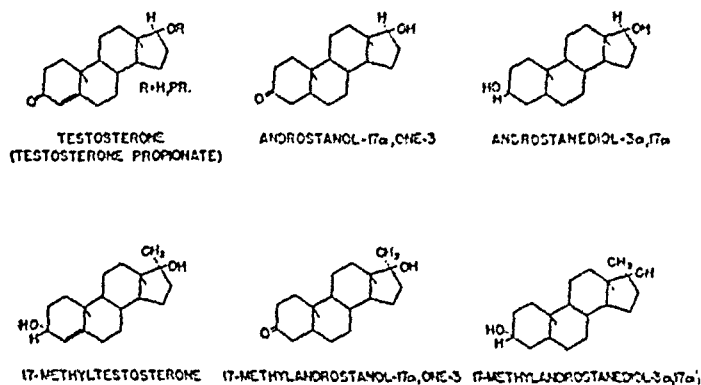


Fig. 6. STERIC CONFIGURATION of the 17-hydroxyl group has been indicated according to the recent revision (14); the designations of  $\alpha$  and  $\beta$ , however, have been maintained since these were introduced (15) in anticipation of such revisions.

these tissues, it has not been recognized as yet. On the other hand, a group of substances in proper combination may be necessary or the available methods of study do not provide an adequate simulation of normal conditions.

The high myotrophic-androgenic ratios produced by the diols recalls the very similar renotrophic-androgenic ratios produced in castrated mice by these steroids (4-6). The different myotrophic-androgenic ratios of the different steroids and their increase or decrease with dose, depending on the particular steroid, tempts speculation as to whether these changes may not be a reflection of the nature of the metabolites formed by the body from the administered steroids. Thus, the diols especially at lower doses would be converted by the body primarily to metabolites which are myotrophic while the testosterone compounds are metabolized primarily to androgenic steroids.

The observation that androstanol-17 $\alpha$ , one-3 is the most potent myotrophic steroid serves to illustrate again that the various properties of the steroids do not parallel each other. Furthermore, it illustrates that a slight alteration in chemical structure (fig. 6) can change a physiological property of a steroid.

The analyses of the muscles indicate that the growth is similar to that present

in normal animals and provides direct support for the many calculations made on the basis of changes in the urinary excretion of constituents necessary for the formation of protoplasm after androgenic stimulation (2, 10).

The much greater efficacy of a steroid when administered by pellet as compared with injection is well illustrated again. Pellets of testosterone propionate were roughly one hundred times as effective as injections.

The failure of castration or any of the steroids to affect the size of the kidney of the guinea pigs is in marked contrast to that observed in the mouse (4-6), but in agreement with the observations in the hamster (11) and the rabbit (12). The hamster, however, is different from the guinea pig (also most other mammals), with respect to body size. The female of this species is larger than the male and has larger skeletal muscles. Castration of the male produces the female type (13).

The ability of testosterone to maintain the temporal muscles at exactly normal size indicates that the demands for restorative processes are much greater than those for maintenance. A similar dose of testosterone was able to restore the atrophied muscle of the castrated guinea pig only to approximately 50 per cent of normal.

#### SUMMARY

Male guinea pigs were castrated at 250 gm. body weight and 35 days later were implanted subcutaneously with pellets of various steroids. After 30 days the animals were autopsied. Castration decreased the temporal muscles to less than one-third that of the normals. The steroids increased the muscles in the following descending order: androstanol-17 $\alpha$ ,one-3; 17-methylandrostanol-17 $\alpha$ ,one-3; testosterone and testosterone propionate; 17-methyltestosterone; androstanediol-3 $\alpha$ ,17 $\alpha$ ; and 17-methylandrostanediol-3 $\alpha$ ,17 $\alpha$ . The dose response was logarithmic for all of the compounds except androstanediol-3 $\alpha$ ,17 $\alpha$ , which became as effective as testosterone at higher dose levels. The myotrophic activities did not parallel the androgenic properties of these steroids. Therefore, the myotrophic-androgenic ratio (increase of muscle weight divided by the increase in accessory sex organs) for the various steroids decreased as follows: androstanediol-3 $\alpha$ ,17 $\alpha$ ; 17-methylandrostanediol-3 $\alpha$ ,17 $\alpha$ ; 17-methylandrostanol-17 $\alpha$ ,one-3; androstanol-17 $\alpha$ ,one-3; testosterone and testosterone propionate; and 17-methyltestosterone.

The temporal muscles were never restored to more than 50 per cent of the normal, but the accessory sex organs were restored. If pellets of testosterone were implanted at time of castration, the muscles were maintained at exactly the same weight as those of normal controls, but the seminal vesicles and prostates were greatly increased. The normal condition was not simulated by any of the steroids. Analyses of the muscles indicated that normal growth occurred under steroid stimulation.

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# EFFECT OF CASTRATION AND STEROIDS ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE GUINEA PIG<sup>1, 2</sup>

JANE HARRISON HUMM,<sup>3</sup> CHARLES D. KOCHAKIAN AND MARY N. BARTLETT

*From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

CERTAIN androgens produce remarkable increases in the arginase activities of the kidney of the mouse (1-3), moderate increases in the kidney of the rat (4) and a decrease in that of the hamster (5). These steroids decrease the 'alkaline' phosphatase of the kidney of the mouse at 'high' doses (6), but produce a moderate increase at 'low' doses (7) and similar increases in that of the rat (4) and hamster (5). The 'acid' phosphatase of the kidney is increased in the mouse (6), but not changed in the rat (4) or the hamster (5). These enzymes of the liver are not changed by administration of androgens in any of the above species.

The above studies now have been extended to another species, the guinea pig.

## PROCEDURE

The guinea pigs were those used in the preceding report (8). At autopsy approximately one gm. of the left section of the median lobe of the liver and one half of one kidney were weighed, placed in 5 ml. of cold redistilled water, homogenized and sufficient water added to make a concentration of 20 ml. of water/gm. of tissue. The enzymes were determined as previously described (1, 2, 4, 6).

## RESULTS

*Kidney arginase.* The normal guinea pigs showed a significantly greater arginase activity than their castrated controls in the 30-day experiments, but this difference disappeared on extension of the time to 90 days (table 1A).

None of the steroids produced a remarkable increase in the arginase of the kidney (table 1A); 17-methyltestosterone and 17-methylandrostanediol-3 $\alpha$ ,17 $\alpha$  produced the greatest increases. The latter steroid, however, was more effective than the former at similar doses. Testosterone was completely ineffective at all dose levels while its propionate produced a small but not significant change at 30 days, which was not apparent at 90 days and was enhanced to a significant increase by the injection of the relatively large dose of 12.5 mg/day for 14 days (table 1B). 17-methylandrostanol-17 $\alpha$ ,one-3 at the higher dose and androstanol-17 $\alpha$ ,one-3 produced small increases in arginase activity. Androstanediol-3 $\alpha$ ,17 $\alpha$  at a similar dose level was ineffective.

Received for publication July 19, 1948.

<sup>1</sup> This investigation was supported by the Josiah Macy, Jr. Foundation.

<sup>2</sup> Parts of these data have been reported in the Josiah Macy Jr. Foundation Conferences on the Metabolic Aspects of Convalescence, 16th meeting, New York, 1947.

<sup>3</sup> Part of these data was taken from the M.S. thesis of Jane Harrison Humm.

*Kidney 'alkaline' phosphatase.* The kidney of the normal guinea pigs demonstrated a higher 'alkaline' phosphatase activity than that of their castrated controls. This difference, however, was not apparent in the older animals (table 1A). All of

TABLE 1. EFFECT OF CASTRATION AND VARIOUS STEROIDS (PELLETS) ON ARGINASE AND 'ALKALINE' PHOSPHATASE OF THE GUINEA PIG KIDNEY<sup>1</sup>

	NO. OF G. PIGS	TOTAL STEROID ABSORBED	KIDNEY WT.	ARGINASE <sup>2</sup>	'ALKALINE' PHOSPHATASE <sup>2</sup>
		mg/30 days	gm.	(U/gm.) %	(U/gm.) %
<i>A. 30-Day Experiments</i>					
Castrated controls	8		3.935	(31) (22-40)	(84) (63-112)
Normal controls	6		4.039	+58	+63
17-Methyltestosterone	5	28.3	4.344	+79	+43
	5	17.7	4.405	+41	+24
	5	6.8	4.319	+40	+39
Testosterone	5	32.2	4.451	-15	+24
	5	18.9	4.513	-3	+42
	5	7.4	4.204	+5	+39
Testosterone propionate	6	7.5	4.296	+27	+36
17-Methylandrostanol-17 $\alpha$ , one-3	5	4.4	4.344	+36	+40
	5	1.8	3.998	+4	+50
Androstanol-17 $\alpha$ , one-3	5	9.1	4.193	+50	+71
	6	5.0	4.499	+33	+41
17-Methylandrostanediol-3 $\alpha$ , 17 $\alpha$	5	9.5	4.168	+73	+29
	4	1.9	3.727	-11	+35
Androstanediol-3 $\alpha$ , 17 $\alpha$	5	5.8	4.023	+22	+19
<i>B. 90-Day Experiments</i>					
Castrated controls	4		5.074	(31) (26-36)	(97) (85-104)
Normal controls	4		4.990	-16	+29
Testosterone propionate	4	20.5	5.117	+10	+28

<sup>1</sup> There was no significant change in the 'acid' phosphatase of the kidney nor in any of the enzymes of the liver.

<sup>2</sup> Average and range of values for the controls are given in parentheses. The % values are differences from the average values of the controls.

the steroids restored in varying degrees the decrease in activity of the phosphatase toward normal. The increases, however, were not related to the dose or the chemical nature of the steroid.

*Kidney 'acid' phosphatase.* The changes in this enzyme were generally less than 5 per cent and never more than 10 per cent. The changes were not significant and are not presented.

*Liver enzymes.* There were no significant changes in the activities of the enzymes of the liver. Most of the differences were below 5 per cent and in a few instances as much as 18 per cent.

#### DISCUSSION

The failure of castration and the steroids to influence any of the liver enzymes is the same as the observations in the mouse (1-3, 6, 7), rat (4) and hamster (5). Thus, in the guinea pig as in the other species the protein anabolic effect of these steroids (9) does not require a change in the activities of these enzymes.

The ability of 17-methyltestosterone to increase the arginase activity of the kidney is apparently due to the 17-methyl group, for testosterone at comparable

TABLE 2. EFFECT OF INJECTION OF A 'LARGE DOSE' OF TESTOSTERONE PROPIONATE (T.P.) ON KIDNEY ENZYMES OF CASTRATED MALE GUINEA PIGS<sup>1</sup>

	NO. OF G. PIGS	DOSE		KIDNEY WT.  gm.	ARGINASE  U/gm.	PHOSPHATASES	
		mg/day	days			'ALKALINE'  U/gm.	'ACID'  U/gm.
Controls	5			4277	29 (19-33)	76 (62-85)	21 (20-23)
T.P.	5	12.5	14	4360	40 (36-45)	79 (70-83)	21 (20-22)
Change %					+38	+4	0

<sup>1</sup> There was no significant change in the enzymes of the liver.

dose levels was completely ineffective. The increase, however, is much smaller than that observed in the mouse (1-3) and even lower than in the rat (4). Furthermore, an attempt to obtain a great increase in arginase activity by the injection of the relatively large dose of 12.5 mg/day of testosterone propionate was unsuccessful. The increase produced was very small and in no manner comparable to that in the mouse (2).

The ability of the steroids to increase the 'alkaline' phosphatase of the kidney is comparable to that noted in the mouse at subnormal doses (7) and in the rat (4) and hamster (5) regardless of dose.

#### SUMMARY

Male guinea pigs were castrated at about 250 gm. body weight. Thirty-five days later they were implanted subcutaneously with pellets of the following steroids: 17-methyltestosterone; testosterone; testosterone propionate; 17-methylandrostanol-17 $\alpha$ ,one-3; androstanol-17 $\alpha$ ,one-3; 17-methylandrostanediol-3 $\alpha$ ,17 $\alpha$ ; and androstanediol-3 $\alpha$ ,17 $\alpha$ . The dose of steroid was varied by the number of pellets implanted. Castration produced a decrease in the arginase activities of the kidney after 60 days, but not after 120 days. None of the steroids produced any remark-

able changes. The greatest increase, 79 per cent, was produced by 17-methyltestosterone while testosterone was completely ineffective. The administration of a relatively large dose, 12.5 mg/day, of testosterone propionate for 14 days produced only a 38 per cent increase. Castration produced a decrease in the 'alkaline' phosphatase of the kidney, which was restored toward normal by the various steroids. None of the enzymes of the liver or the 'acid' phosphatase of the kidney were affected by castration or the steroids.

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# EFFECT OF TESTOSTERONE PROPIONATE AND GROWTH HORMONE ON THE WEIGHTS AND COMPOSITION OF THE BODY AND ORGANS OF THE MOUSE<sup>1, 2</sup>

CHARLES D. KOCHAKIAN AND CONSTANCE E. STETTNER

*From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

THE steroid N-hormones, e.g. testosterone (1) and the growth hormone of the anterior pituitary (2-4), are very potent stimulators of protein anabolism. It seemed of importance, therefore, to make a comparison of the sites and nature of the growth stimulated by these two important hormones.

## PROCEDURE

*Animals.* White male mice of the Swiss strain were castrated at 17- to 19-gm. body weight; treatment was begun 30 to 40 days later. They were maintained in a glass jar containing wood shavings in an air-conditioned room at 78 to 80°F. They were fed the Rockland rat diet and tap water *ad libitum*. Body weights were recorded three times per week beginning one week before treatment.

*Hormones.* The testosterone propionate<sup>3</sup> was implanted subcutaneously as a pellet of approximately 14 mg. (5). The growth hormone extract was prepared from beef pituitary. It contained "1.1 units/mg. in the 6-month old 'plateaued' female rat growth test"<sup>4</sup> and "in the hypophysectomized immature female rat,<sup>5</sup> 0.1 mg/day for 10 days produced an average increase of 14.1 gm. in body weight. It showed no corticotrophin activity but at a total dose of 2.5 mg. it repaired the interstitial cells of the ovary and the thyroid of the hypophysectomized rat".

The extract was supplied as a dry powder and was dissolved as follows: 100 mg. was placed in a 15-ml. centrifuge tube set in an ice water bath, 8 ml. of ice cold N/100 sodium hydroxide was added and the suspension vigorously stirred at intervals for 30 to 60 minutes. Then the mixture was titrated, to pH 9 (Universal indicator, Eastman Kodak Co.) with N/50 phosphoric acid. It was made to 10 ml. with distilled water, stirred and centrifuged. The supernatant solution was decanted into rubber-capped vials and kept at 0 to 5°C. A maximum of one week's supply was prepared at one time. It was injected at 0.1 ml. (1.1 U.) per day.

*Autopsy and preparation of tissues.* The food was removed 18 to 24 hours before the end of the experiment. The mice were killed by severing the spinal chord at the base of the skull and bleeding from the blood vessels of the neck. The organs were removed and weighed on a Roller-Smith torsion balance. The kidneys and livers were homogenized (6) and aliquots removed for enzyme (7) and nitrogen determinations. The gastro-intestinal tract was freed of fecal material and the carcass and contents were dried to constant weight at 80 to 90°C. in an electric oven attached to a water aspirator. The dried residue was dissolved with warming in 30 ml. of 50 per cent potas-

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Received for publication August 5, 1948.

<sup>1</sup> This investigation was supported by the Josiah Macy Jr. Foundation and Ciba Pharmaceutical Products Inc.

<sup>2</sup> The data in this paper were presented before the American Physiological Society, Atlantic City, March 1948. (*Federation Proc.* 7(1): 66, 1948).

<sup>3</sup> The testosterone propionate (perandren) was provided by Ciba Pharmaceutical Products Inc.

<sup>4</sup> The growth-hormone powder, R 70832, was provided and assayed by Parke, Davis and Co. through the courtesy of Drs. D. A. McGinty and L. W. Donaldson.

<sup>5</sup> These assays were performed by the Institute of Experimental Biology, Berkeley, Calif. through the courtesy of Dr. C. H. Li.



sium hydroxide and 30 ml. of redistilled alcohol and transferred to a 200-ml. volumetric flask. The bones were dissolved in 3 N hydrochloric acid. The mixture then was made to volume with distilled water.

The seminal vesicles and prostates and the remainder of the kidney and liver homogenates were pooled in separate groups of 3 to 5, dried to constant weight as above, dissolved in 10 ml. of 50 per cent potassium hydroxide and 10 ml. of redistilled alcohol and made to 50 ml.

*Nitrogen determination.* The micro-Kjeldahl procedure was used for all nitrogen determinations except that aliquots of the dissolved carcasses were digested by the macro procedure, made to a given volume from which samples were distilled by the micro procedure.

*Total fat determination.* A modified Leathes and Raper method (8, cf. 9) was used. An aliquot of the dissolved tissue was pipetted into a 250-ml. centrifuge bottle containing crushed ice, an equal volume of 6 N hydrochloric acid was added and the mixture extracted three times with 75 ml. of distilled petroleum ether (Skellysolve F). The pooled extract was washed once with 50 ml. of 40 per cent alcohol which was in turn extracted twice with fresh petroleum ether. The bottles were centrifuged after each extraction and the Skellysolve F removed by aspiration. The extracts were combined, concentrated to about 10 ml., transferred to a tared 50-ml. erlenmeyer flask, evaporated to dryness and placed in a vacuum desiccator containing 'dehydrite' for at least 24 hours before weighing.

## RESULTS AND DISCUSSION

*Body weight.* The chosen doses of testosterone propionate and the growth hormone extract produced similar increases in the body weight except that the rate of increase was slightly different (fig. 1). The mice treated with the androgen showed an initially more rapid rate of increase, which decreased to less than that of the growth-hormone treated mice after about seven days of treatment. The growth curves of the 10- and 20-day treated mice were similar to the respective portions of the growth curves of the 34-day treated mice (fig. 1).

*Organ weights.* The change in body weight was due in part to changes in organ weights. Testosterone propionate produced the expected increase in the accessory sex organs. The growth hormone was ineffective when given alone or simultaneously with testosterone propionate (table 1). Testosterone propionate produced a progressive increase in the size of the kidneys with duration of treatment. The growth hormone produced a much smaller increase in this organ and no further increase was obtained on extending the period of injections. The simultaneous administration of the hormones produced a partial summation of their renotropic effects.

There was a small increase (table 1) in the size of the liver in all of the mice except those treated with the androgen for 10 days. There was no evidence of summation when the two hormones were administered simultaneously. The androgens decreased the size of the thymus but the growth hormone was ineffective. There was no gross evidence of adrenal changes.

*Composition of the organs.* There was no major change in the percentage composition of the kidney or the liver (table 2) as a result of the various treatments. The seminal vesicles and prostates (table 3) had approximately the same percentage composition of water and protein in the various groups, but the fat content was decreased in the animals treated for 34 days. The organs of the control animals were too small (cf. table 1) to permit analyses.

*Composition of the carcass.* The water content of the carcass was progressively increased by both hormones. The increases, however, completely disappeared in the

10-day treated mice and become very small in the longer treated animals when the calculations are made on a fat-free basis (cf. 10, 11). The increases, nevertheless, are always greater after growth-hormone than androgen treatment and there is also a

Fig. 1. EFFECTS OF (TESTOSTERONE PROPIONATE (T.P.) and growth hormone (G.H.) on body wt. of castrated mice.

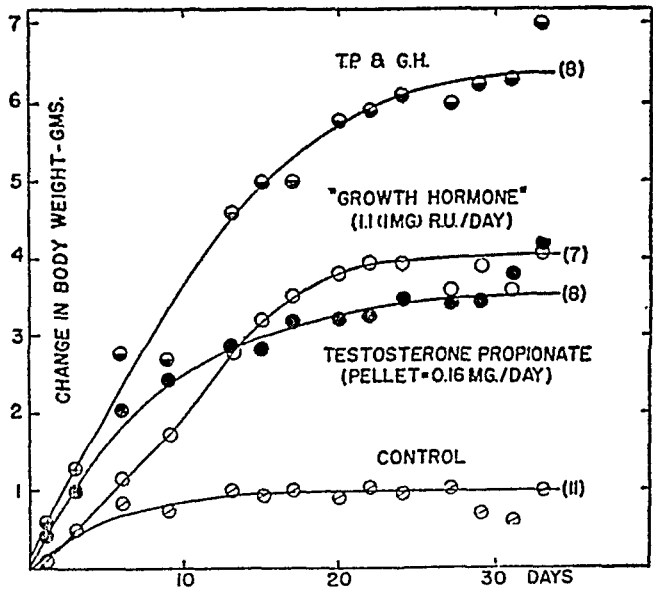


TABLE I. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND AN ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON THE ORGAN WEIGHTS OF CASTRATED MICE

			NO. OF MICE	SEM. VES. + PROS.	KIDNEYS	LIVER	THYMUS
	mg/day	days		(mg), % <sup>1</sup> (10) (5-14)	(mg), % <sup>1</sup> (234) (193-260)	(mg), % <sup>1</sup> (920) (720-1010)	(mg), % <sup>1</sup> (47) (34-71)
Controls			19				
T. P.	0.15	10	6	1600	57	4	-60
	0.16	20	9	2900	73	20	-95
	0.15	34	8	3500	81	15	-95
G. H.	1.0	10	9	30	37	29	+6
	1.0	20	9	10	30	35	+7
	1.0	34	7	30	24	11	-18
T. P. and G. H.	{ 0.16 1.0 0.16 1.0	20	8	3000	93	31	-95
		34	8	4200	91	20	-95

<sup>1</sup> Percentage difference from the control values; the averages and range of values are given as milligrams in parentheses.

suggestion of partial summation when both hormones are administered. The nitrogen content was not remarkably altered. The quantity of fat was decreased by both hormones but the growth hormone was more effective than the androgen.

*Composition of the increase in body weight.* A summary of the total changes in all

of the organs and carcass is presented in figure 2. The increase in body weight over that of the controls occurred as a result of protein synthesis. Furthermore, when the two hormones were administered simultaneously there was a summation of their protein anabolic properties accompanied by a loss in body fat, which occurred entirely in the carcass (table 4). Indeed the organs, especially the seminal vesicles and prostates, synthesized fat in proportion to their increases in weight (tables 1-3). The

TABLE 2. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) ON COMPOSITION OF THE KIDNEY AND LIVER OF THE CASTRATED MOUSE

	NO. OF MICE	KIDNEY			LIVER		
		Water	Nitrogen	Fat	Water	Nitrogen	Fat
		%	%	%	%	%	%
Controls.....	13	73.8	3.03	3.9	67.0	3.50	5.5
<i>10-Day Experiments</i>							
T. P.....	6	74.5	2.95	3.4	65.6	3.57	6.1
G. H.....	9	75.2	2.94	3.8	66.2	3.54	4.8
<i>20-Day Experiments</i>							
T. P.....	9	74.6	3.03	3.3	66.8	3.67	4.3
G. H.....	9	74.8	3.12	3.3	67.1	3.58	4.5
T. P. + G. H.....	8	74.8	3.02	3.2	67.1	3.70	4.3
<i>34-Day Experiments</i>							
T. P.....	8	74.2	2.89	3.7	65.4	3.94	4.6
G. H.....	7	74.8	2.96	3.8	68.8	3.63	3.8
T. P. + G. H.....	8	75.8	3.00	3.0	67.3	3.86	4.2

TABLE 3. EFFECT OF TESTOSTERONE PROPIONATE (T. P.) ALONE AND WITH GROWTH HORMONE (G. H.) ON COMPOSITION OF THE SEMINAL VESICLES AND PROSTATES OF CASTRATED MICE<sup>1</sup>

	10 DAYS			20 DAYS			34 DAYS		
	Water	Nitrogen	Fat	Water	Nitrogen	Fat	Water	Nitrogen	Fat
	%	%	%	%	%	%	%	%	%
T. P.....	73.8	3.10	3.9	71.7	3.60	3.4	73.5	3.41	2.0
T. P. + G. H.....				71.8	3.40	4.2	72.7	3.50	2.5

<sup>1</sup> The seminal vesicles and prostates of the control and growth-hormone-injected mice were too small (cf. table 1) to permit analyses.

amounts, however, are relatively insignificant when compared to the changes in the carcass.

In every instance the greatest synthesis of protein occurred in the carcass (table 5) and became greater with increase in duration of treatment. The amount, however, was less in the androgen- than in the growth-hormone-treated mice because of the greater synthesis of protein in the kidney and especially the seminal vesicles and prostates.

The effect of the growth hormone on the body and organ weights and their composition is in general agreement with previous studies in the mouse (9, 12) and

Fig. 2. COMPOSITION OF THE INCREASE IN BODY WT. of castrated mice treated with testosterone propionate (T. P.) and growth hormone (G. H.) over that of control animals.

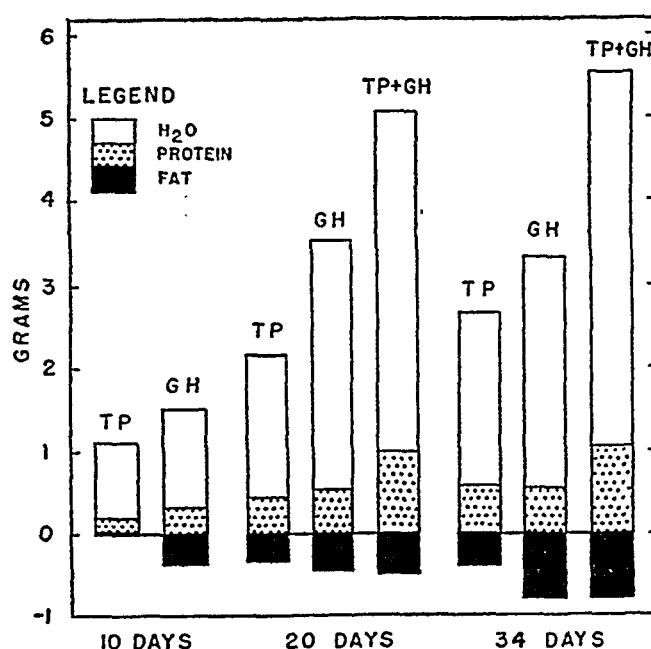


TABLE 4. EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) ON COMPOSITION OF THE CARCASS OF CASTRATED MICE

	NO. OF MICE	WATER	NITROGEN	FAT	FAT FREE BASIS	
					Water	Nitrogen
10-Day Experiments						
Control.....	7	% 61.9	% 2.91	% 12.6	% 70.5	% 3.41
T. P.....	6	62.3	2.88	10.9	69.5	3.23
G. H.....	9	63.8	2.94	10.3	71.5	3.23
20-Day Experiments						
Control.....	7	62.1	2.91	12.6	70.5	3.41
T. P.....	8	63.9	2.93	10.2	71.4	3.27
G. H.....	9	65.3	2.82	9.2	72.1	3.11
T. P. + G. H.....	8	66.5	2.95	8.4	72.4	3.22
34-Day Experiments						
Control.....	5	61.8	2.91	12.6	71.2	3.37
T. P.....	8	64.4	3.04	9.8	72.0	3.37
G. H.....	7	67.7	3.02	7.8	72.8	3.24
T. P. + G. H.....	8	67.8	3.04	7.2	73.6	3.29

the rat (3, 13, 14). There are, however, contradictory reports concerning the effect of this hormone on the liver and kidney weights of the rat (cf. 15).

The chosen doses of the two hormones fortunately produced nearly identical increases in body weight. Thus a comparison of the results of the two types of treat-

ment are greatly facilitated. The effect of these two hormones are distinctly different. The growth hormone as expected had no androgenic effect in contrast to the testosterone propionate. The renotropic properties also were decidedly different. The androgen not only produced larger kidneys but also progressively increased the size with extension of treatment, while the growth hormone produced only a small increase in kidney weight which was not increased further by extending the duration of treatment.

The simultaneous administration of the two hormones produced almost an exact summation of increase in body weight and a partial summation of their renotropic effects. It is unlikely that these results are due to a further stimulation of the same intermediary metabolic processes, for doubling the dose of testosterone propionate does not produce a further increase in the body weight of the castrated mouse. There is, however, a slight increase in the size accompanied by a further increase in the arginase activity of the kidney (16). On the other hand, the growth hormone inhibits the increase in arginase activity of the kidney produced by testosterone propionate (7).

TABLE 5. SITES AND RELATIVE AMOUNT OF PROTEIN ANABOLISM INDUCED BY TESTOSTERONE PROPIONATE (T. P.) AND GROWTH HORMONE (G. H.) IN CASTRATED MICE

	10 DAYS		20 DAYS			34 DAYS		
	T. P.	G. H.	T. P.	G. H.	T. P. + G. H.	T. P.	G. H.	T. P. + G. H.
	%	%	%	%	%	%	%	%
Carcass.....	69.2	76.7	65.3	80.4	79.4	75.5	92.3	83.0
Liver.....	2.8	19.5	10.6	16.8	9.3	8.7	6.2	5.5
Kidney.....	11.3	4.5	7.1	2.8	4.3	4.7	1.5	3.4
Seminal vesicles and prostates.....	16.7	0.0	16.8	0.0	7.0	11.1	0.0	8.1

Values are percentages of the increase in body weight (see fig. 1).

The total composition of the increase in body weight is in general the same for the two hormones. There was an increase in protein synthesis and an increase in fat catabolism. The amount of fat catabolism, however, was greater and occurred sooner in the growth-hormone than in the androgen-treated mice. Furthermore, there was no apparent summation of this effect, while there was in protein synthesis when the two hormones were administered simultaneously. The increase in fat catabolism agrees with the decreases in R. Q. observed after administration of growth hormone (17) and androgens (1, 18) to dogs.

The site of greatest protein anabolism and all of the fat catabolism occurred in the 'carcass'. The kidney, liver and especially the seminal vesicles and prostates of the androgen treated mice were sites of not only protein but also fat synthesis. The protein synthesized in the carcass was similar to that in young growing animals (10) especially in the growth-hormone-treated mice. The composition of the kidney and liver was the same as that in the control animals.

The stimulation of protein synthesis in the carcass by the androgen presumably occurred in the skeletal muscles. These tissues of the castrated guinea pig are in-

creased in size by testosterone propionate (19) and related steroids (20). The skin also may have either participated or detracted from the changes noted since its hair growth and appearance is altered by androgen injections (21).

#### SUMMARY

White male mice were castrated at 17 to 19 gm. body weight and approximately one month later, separate groups were treated as follows: *a*) injected daily with 1.1 rat growth units (1 mg.) of anterior pituitary growth hormone, *b*) implanted subcutaneously with an approximately 14-mg. pellet of testosterone propionate and *c*) both treatments simultaneously. The mice treated with the growth-hormone preparation showed approximately the same increase in body weight as those treated with the androgens. When the two hormones were administered simultaneously, there was a summation effect on the increase in body weight.

The growth hormone produced a small increase in kidney weight which was not further enhanced by extending the period of treatment. The androgen on the other hand, produced a much greater increase in this organ which was further increased on extending the period of treatment. The effects on the kidney were partially summated when the two hormones were administered simultaneously.

Both hormones increased the total amount of protein and water in the carcass and the organs and decreased the fat of the carcass. The increased amount of protein was similar for both hormones, but the androgen diverted a larger proportion to the kidneys and especially the seminal vesicles and prostates. The growth hormone caused a slightly greater increase in water content and a much greater decrease in fat content of the carcass than the androgen. The simultaneous administration of the two hormones produced a summation of the protein anabolic but not the fat catabolic effects.

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# EFFECT OF TESTOSTERONE PROPIONATE AND GROWTH HORMONE ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE MOUSE<sup>1, 2</sup>

CHARLES D. KOCHAKIAN AND CONSTANCE E. STETTNER

*From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

IN PREVIOUS reports it has been demonstrated that testosterone propionate increases the arginase (1-3) and to a lesser extent the 'acid' phosphatase, but decreases the 'alkaline' phosphatase (3-5) of the mouse kidney. These enzymes of the liver are not affected. Since the growth hormone of the anterior pituitary also is a very potent stimulator of protein anabolism (6, 7), it seemed worth while to determine whether this hormone also affected the above enzymes.

## PROCEDURE

The mice were those used in the preceding report (8). The enzymes were determined as previously described (1, 2, 5).

## RESULTS

*Kidney enzymes.* The testosterone propionate<sup>3</sup> produced the expected marked increase in arginase (1, 2), the small increase in 'acid' and the marked decrease in 'alkaline' phosphatases (5) (table 1). The arginase and 'alkaline' phosphatase showed no further changes when the androgen treatment was extended to 20 and 34 days. Growth hormone,<sup>4</sup> on the other hand, did not significantly alter the concentration of the enzyme activities, except in the case of the kidney arginase which was moderately increased only in the mice injected for 10 days.

The simultaneous administration of growth hormone with testosterone propionate markedly reduced the ability of the androgen to increase the arginase activity of the kidney, but did not significantly alter the effect on the phosphatases.

*Liver enzymes.* The hormones either separately or simultaneously administered did not alter the concentration of the various enzymes (table 2).

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Received for publication August 5, 1948.

<sup>1</sup> This investigation was supported by the Josiah Macy Jr. Foundation.

<sup>2</sup> The data in this paper were presented before the American Physiological Society, Atlantic City, March 1948 (*Federation Proc.* 7(1): 66, 1948).

<sup>3</sup> The testosterone propionate was supplied as crystalline material by Ciba Pharmaceutical Products Inc.

<sup>4</sup> The anterior pituitary growth-hormone preparation, R 70832, was supplied as a dry powder by Parke, Davis and Company.

TABLE 1. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON ENZYMES OF THE KIDNEY OF CASTRATED MICE

	NO. OF MICE	HORMONE		KIDNEYS	ARGINASE	PHOSPHATASES	
						'Alkaline'	'Acid'
		days	mg/day	mg.	(U/gm.) <sup>1</sup> %		
Control	19			234	50 (30-84)	336 (246-400)	10.3 (8.8-12.9)
T. P. <sup>2</sup>	6	10	0.15	367	+268	-48	0
	9	20	0.16	404	+202	-54	+10
	8	34	0.15	423	+202	-48	+15
G. H.	9	10	1.0	320	+52	+8	+2
	9	20	1.0	304	0	-3	+6
	7	34	1.0	290	+14	-3	+11
T. P. <sup>2</sup> + G. H.	8	20	0.16 1.0	464	+100	-37	+14
	8	34	0.16 1.0	448	+114	-45	+21

<sup>1</sup> The changes are percentage differences from the control values, which are given in parentheses with the ranges.

<sup>2</sup> Implanted subcutaneously as a 14- to 15-mg. pellet.

TABLE 2. COMPARISON OF EFFECT OF TESTOSTERONE PROPIONATE (T. P.) AND ANTERIOR PITUITARY GROWTH HORMONE (G. H.) ON ENZYMES OF THE LIVER OF CASTRATED MICE

	NO. OF MICE	HORMONE		LIVER	ARGINASE	PHOSPHATASES	
						'Alkaline'	'Acid'
		days	mg/day	gm.	(U/gm.) <sup>1</sup> %		
Control	19			0.92 (0.72-1.01)	(16,800) (14,300-18,500)	(8.1) (6.9-9.6)	(18.9) (17.7-20.2)
T. P. <sup>2</sup>	6	10	0.15	0.96	-3	+18	-3
	9	20	0.16	1.04	+8	+5	-13
	8	34	0.15	1.01	+10	+24	-4
G. H.	9	10	1.0	1.19	+10	-2	+18
	9	20	1.0	1.29	+22	+7	+8
	7	34	1.0	1.02	-4	+3	-3
T. P. <sup>2</sup> + G. H.	8	20	0.16 1.0	1.24	+5	+26	+15
	8	34	0.16 1.0	1.10	+1	+32	+2

<sup>1</sup> The changes are percentage differences from the control values which are given in parentheses with the ranges.

<sup>2</sup> Implanted subcutaneously as a 14- to 15-mg. pellet.



## DISCUSSION

It is evident that neither testosterone propionate nor growth hormone decreases the requirement of arginase in the liver while stimulating protein anabolism. On the contrary as the liver is stimulated to increase in size there is a proportionate increase in the enzyme activity. It is impossible at present to reconcile these results with the reported (8) decrease in arginase activity of the liver of the hypophysectomized rat, especially since the decreases observed in either hypophysectomized or normal adult male rats in this laboratory (unpublished) range from 0 to 20 per cent. It is of interest that the 'alkaline' and 'acid' phosphatase activities also increase in proportion to the increase in liver protein.

The two hormones do not show the same effect in their actions on the enzymes of the kidney. The action of the growth hormone is identical to that on the liver. There is a proportionate increase in the enzymes with kidney mass. The androgen, on the other hand, produces a very great increase in the arginase, a small increase in the 'acid' phosphatase and a marked decrease in the 'alkaline' phosphatase activities. Furthermore, the effect of the androgen on the arginase activity is greatly inhibited while the phosphatases are not affected by the simultaneous administration of the growth hormone. Thus, there is a definite difference in the mechanism of the intermediary metabolic processes stimulated by these two protein anabolic hormones. Indeed, there is even a suggestion of antagonism or competition.

## SUMMARY

Mice were castrated at 17- to 19-gm. body weight and one month later were implanted subcutaneously with 1) a 14- to 15-mg. pellet of testosterone propionate, 2) injected subcutaneously with 1.1 rat U/day of growth hormone and 3) treated simultaneously with both hormones for 10-, 20- and 34-day periods. Testosterone propionate produced the expected marked increase in kidney arginase, small increase in 'acid' (pH 5.4) phosphatase and marked decrease in 'alkaline' (pH 9.8) phosphatase activities. Growth hormone was ineffective but when administered simultaneously with testosterone propionate, it decreased the arginase-stimulating effect of the androgen to one-half.

The liver enzymes were not affected by either of the hormones. The small increases in liver size were accompanied by proportionate increases in the enzyme activities.

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# EFFECT OF ESTROGEN ALONE AND IN COMBINATION WITH TESTOSTERONE ON THE BODY AND ORGAN WEIGHTS AND THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE MOUSE<sup>1</sup>

CHARLES D. KOCHAKIAN, E. E. GARBER AND MARY N. BARTLETT

*From the Department of Physiology and Vital Economics, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

**I**N PREVIOUS studies the effect of testosterone (1) and  $\alpha$ -estradiol (2) on the organ weights and enzymes of the castrated mouse have been reported. In this study, the effects of the combination of these two hormones and also the androgen with methoxybisdehydrodoisynolic acid are being reported. In addition studies with two new synthetic estrogens are included.

## PROCEDURE

Male mice were castrated at 17- to 19-gm. body weight; one month later the pellets of the various steroids<sup>2</sup> were implanted subcutaneously (3). When both estrogen and androgen were implanted, they were inserted at different sites. Mice of the dba<sup>3</sup> strain were used in all of the studies except at the lower dose for the 16-day experiments and for the studies with 1-methylestrone and 1-methylestradiol for which mice of the Swiss strain from our Bacteriology department were used. This change was necessitated because the dba mice were no longer available.

The food, Rockland rat diet, was fed *ad libitum* and was removed from the cages the day before autopsy. The mice were killed by severing the spinal chord at the base of the skull and bleeding by cutting the blood vessels of the neck. The organs were removed and weighed on a Roller-Smith torsion balance. The weighed kidneys and liver were homogenized and the enzyme activities determined as previously described (3-6).

## RESULTS

*Rate of absorption of the estrogens.* Methoxybisdehydrodoisynolic acid (hereafter designated as MDDA) was absorbed at approximately eight times the rate of  $\alpha$ -estradiol (table 2). The solubility of the estrogens or the androgen was not noticeably altered by simultaneous implantation (tables 1, 2). The difference in solubility between the two estrogens was greatly reduced when they were mixed with cholesterol. The MDMA had only about twice the rate of absorption of  $\alpha$ -estradiol.

The introduction of the methyl group into  $\alpha$ -estradiol, 1-methylestradiol, increased the solubility of the compound approximately fourfold. Similar alteration of estrone, 1-methylestrone, did not produce the same result. It was ineffective (cf. 2).

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Received for publication August 5, 1948.

<sup>1</sup> This investigation was supported by grants from Ciba Pharmaceutical Products Inc.

<sup>2</sup> The steroids were provided by Ciba Pharmaceutical Products Inc.

<sup>3</sup> These mice were generously provided by the Biological Station, Springville, N. Y., through the courtesy of Dr. S. G. Warner.

TABLE 1. EFFECT OF TESTOSTERONE AND  $\alpha$ -ESTRADIOL ON BODY AND ORGAN WEIGHTS OF CASTRATED MICE

	NO. OF MICE	STERIOD ABSORBED	BODY WEIGHT		SEM. + PROS.	KIDNEYS	THYMUS
			Final Change				
10-Day Experiments							
		mg.	gm.	gm.	(mg.), % <sup>1</sup>	(mg.), % <sup>1</sup>	(mg.), % <sup>1</sup>
Control.....	28		21.5	0.9	(11) (7-13)	(263) (222-295)	(33) (22-53)
Testosterone.....	9	3.3	23.6	2.7	1130	60	-61
Testosterone + $\alpha$ -estradiol.....	5	3.2, 0.7	21.1	0.6	736	58	-61
$\alpha$ -Estradiol.....	5	0.6	22.0	1.3	191	20	-49
30-Day Experiments							
Control.....	28		22.3	1.7	(11) (7-13)	(263) (222-295)	(33) (22-53)
Testosterone.....	9	8.3	24.6	4.4	2860	108	-91
Testosterone + $\alpha$ -estradiol.....	1 <sup>2</sup>	7.0, 2.4	20.7	2.4	1690	105	-99
$\alpha$ -Estradiol.....	6	2.6	20.0	0.1	54	15	-70

<sup>1</sup> The percentage differences are from the average values of the controls which are given with their ranges in parentheses.

<sup>2</sup> Two mice died at 28 days. The weights of their organs were similar to those of the survivor.

TABLE 2. EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON BODY AND ORGAN WEIGHTS OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	PELLETS	STERIOD ABSORBED	BODY WEIGHT		SEM. VES. + PROS.	KIDNEYS	LIVER	THYMUS
				Final change					
			mg/16 days	gm.	gm.	(mg.), %	(mg.), %	(mg.), %	(mg.), %
Control (dba)	5			23.1	1.4	(16) (14-18)	(257) (232-283)	(1030) (890-1090)	(42) (27-54)
Control (Swiss)	9			23.3	1.5	(12) (8-16)	(250) (217-281)	(960) (760-1060)	(51) (34-73)
Testosterone	5	I	7.0	23.1	2.2	1180	73	-9	-93
	5	I	6.3	24.8	2.8	2310	53	-4	-85
Testosterone + $\alpha$ - estradiol	5	I, I	6.6, 0.54	21.3	0.4	800	76	-9	-95
	5	I, I + 3	7.0, 0.05	26.0	2.8	1970	68	11	-92
$\alpha$ -Estradiol	5	I	.61	20.4	-1.4	0	6	-4	-81
	5	I + 3	.06	24.8	3.2	150	5	7	-71
Testosterone + MDDA	2 <sup>1</sup>	I, I	5.6, 4.6	15.6	-4.5	375	10	-15	-95
	5	I, I + 3	6.9, 0.12	24.2	1.4	1740	49	-3	-99
MDDA	5	I	4.8	15.1	-5.5	-37	-32	-27	-99
	5	I + 3	0.10	23.4	1.4	41	8	10	-55
r-Methylestrone	4	I	0.33	21.4	0.4	0	-11	-4	0
r-Methylestradiol	4	I	2.2	22.0	1.6	25	4	0	0

<sup>1</sup> Three mice died before the end of the experiment.

*Body weight.* The mice implanted with pure  $\alpha$ -estradiol and MDDA either gained less weight than their controls or lost weight (table 2). The loss in body weight increased with the amount of estrogen absorbed. Thus, MDDA, which was most rapidly absorbed, produced the greatest decrease in body weight. The reduction of the amount of estrogen absorbed by mixture with cholesterol (table 2), permitted  $\alpha$ -estradiol to increase the body weight. The MDDA, at the lower dose level, which was, however, twice that of  $\alpha$ -estradiol, only maintained the body weight at that of the controls. The simultaneous administration of testosterone exacerbated the urinary retention. Furthermore, 2 out of 3 mice treated for 30 days with testosterone and  $\alpha$ -estradiol (table 1) died on the 28th day and 2 out of 4 mice treated for 16 days with testosterone and MDDA died on the day before and the last day of the experiment. One of these had hemorrhage in the large intestine. Moreover, 3 of the mice treated with testosterone and the low dose of  $\alpha$ -estradiol (table 2) and 2 treated with testosterone and the low dose of the MDDA had markedly distended bladders at autopsy. The controls and the remainder of the experimental mice had negligible amounts of urine in their bladders.

TABLE 3. EFFECT OF TESTOSTERONE AND  $\alpha$ -ESTRADIOL ON THE ARGINASE ACTIVITY OF THE KIDNEY OF CASTRATED MICE

	10-DAY EXPERIMENTS		30-DAY EXPERIMENTS	
	No. of mice	Arginase (U/gm.), %	No. of mice	Arginase (U/gm.), %
Control.....	20	(49) (39-59)	20	(49) (39-59)
Testosterone.....	6	+490	6	+584
Testosterone + $\alpha$ -estradiol.....	5	+760	1	+789
$\alpha$ -Estradiol.....	5	+192	5	+88

*Organ weights.* The effect of testosterone on the seminal vesicles and prostates was greatly decreased by the simultaneous administration of both  $\alpha$ -estradiol and MDDA in all of the experiments (tables 1, 2). The greatest inhibition was observed at the high dose of MDDA (table 2). MDDA and  $\alpha$ -estradiol demonstrated a small and the methylated estrogens no renotrophic properties (tables 1, 2). The renotrophic effect of testosterone was not influenced by the simultaneous administration of either  $\alpha$ -estradiol or MDDA. The small decrease in liver weight produced by testosterone is equivocal. In other studies no change (7) or an increase (unpublished) has been noted (also cf. 8). The high dose of MDDA decreased the weight of the liver which was partially counteracted by testosterone (table 2). The other experiments showed no or questionable small changes which roughly paralleled the changes in body weight. Testosterone was more effective than the estrogens in diminishing the weight of the thymus. The methylated estrogens were completely ineffective in this respect (tables 1, 2).

*Kidney enzymes.* Testosterone as expected (1, 3, 4) greatly increased the arginase activity of the kidney (tables 3, 4). The  $\alpha$ -estradiol and MDDA (tables 3, 4), but not the methylated estrogens (table 4), also increased this enzyme but not as

TABLE 4. EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON ENZYMES OF THE KIDNEY OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	STERIOD ABSORBED  mg/16 days	KIDNEYS mg.	ARGINASE  (U/gm.), %	PHOSPHATASES		NITROGEN  (mg/gm.), %
					'Alkaline'  (U/gm.), %	'Acid'  (U/gm.), %	
Control (dba)	5		257	(63) (32-93)	(376) (350-448)	(9.4) (8.1-10.9)	(31.5) (30.6-32.7)
Control (Swiss)	9		250	(33) (12-70)	(391) (270-480)	(9.5) (7.8-10.5)	(29.8) (28.7-30.8)
Testosterone	5	7.0	444	417	-76	3	-5
	5	6.3	381	728	-61	11	-2
Testosterone + $\alpha$ -estradiol	5	6.6, 0.54	453	750	-71	-2	-8
	5	7.0, 0.05	419	839	-55	-1	-4
$\alpha$ -Estradiol	5	0.61	275	35	-26	3	-3
	5	0.06	262	155	-3	5	+1
Testosterone + MDDA	2	5.6, 4.6	282	855	-68	-9	-9
	5	6.9, 0.12	373	903	-48	6	-5
MDDA	5	4.8	176	16	-6	11	4
	5	0.10	271	167	-2	0	-1
1-Methylestrone	4	.33	221	-1	-5	-1	0
1-Methylestradiol	4	2.2	260	4	-5	6	-2

TABLE 5. EFFECT OF TESTOSTERONE AND VARIOUS ESTROGENS ON ENZYMES OF THE LIVER OF CASTRATED MICE (16-DAY EXPERIMENTS)

	NO. OF MICE	STERIOD ABSORBED  mg/16 days	LIVER mg.	ARGINASE  (U/gm.), %	PHOSPHATASES		NITROGEN  (mg/gm.), %
					'Alkaline'  (U/gm.), %	'Acid'  (U/gm.), %	
Control (dba)	5		1030	(19800) (16400-22800)	(8.9) (4.8-14.5)	(13.8) (11.0-14.3)	(35.6) (33.1-39.2)
Control (Swiss)	9		960	(17900) (12400-21600)	(8.2) (5.1-10.5)	(13.3) (10.0-14.8)	(33.3) (31.9-34.8)
Testosterone	5	7.0	940	-2	14	4	6
	5	6.3	920	-15	17	9	8
Testosterone + $\alpha$ -estradiol	5	6.6, 0.54	940	-2	52	1	3
	5	7.0, 0.05	1060	-8	16	6	16
$\alpha$ -Estradiol	5	0.61	990	18	92	11	2
	5	0.06	1020	5	16	7	5
Testosterone + MDDA	2	5.6, 4.6	870	152	189	51	-2
	5	6.9, 0.12	920	15	60	20	8
MDDA	5	4.8	760	126	225	44	-6
	5	0.10	1050	27	52	4	5
1-Methylestrone	4	0.33	920	3	-21	6	8
1-Methylestradiol	4	2.2	960	16	-5	9	2

greatly as the androgen. Furthermore,  $\alpha$ -estradiol in contrast to testosterone became less effective with continuation of treatment. The greatest increase occurred

after 10 days of treatment (table 3). The simultaneous administration of the androgen and the estrogens always resulted in a summation of their arginase-stimulating properties (tables 3, 4).

Testosterone produced the expected decrease in concentration of 'alkaline' and no effect in 'acid' phosphatase activities (table 2). The total activity of the latter, however, was increased in proportion to the increase in kidney weight. The estrogens were ineffective except for the small decrease produced at the high dose of  $\alpha$ -estradiol. Furthermore, the estrogens did not influence the effect of the androgen on these two enzymes.

*Liver enzymes.* MDDA administered singly or with testosterone (table 5) produced a remarkable increase in the arginase activity at the high dose level and a much smaller increase at the low dose level. No noteworthy changes were evident in the rest of the experiments. The 'alkaline' phosphatase activity also was remarkably increased by MDDA and to a lesser degree by  $\alpha$ -estradiol. At the low dose level the MDDA was much less effective and  $\alpha$ -estradiol was ineffective. The 'acid' phosphatase was moderately increased only by MDDA at the high dose. The methylated estrogens did not influence the activities of any of the enzymes.

*Kidney and liver nitrogen.*<sup>4</sup> There were no remarkable changes in the nitrogen (protein) concentration of either the kidney or the liver in any of the experiments (tables 4, 5). In general the change in organ weight was accompanied by a proportionate change in protein content.

#### DISCUSSION

The opening of the 5 ring in  $\alpha$ -estradiol and the addition of the methoxy group to produce MDDA greatly increased its rate of absorption with a concomitant increase in its physiological effects. The introduction of a methyl group in the one position also increased the rate of absorption of  $\alpha$ -estradiol, but very greatly reduced its physiological properties. A similar change in the estrone molecule, however, did not change its rate of absorption but did greatly reduce its physiological effects. It would seem that the phenolic A ring plays a very important part in the physiological properties of the estrogens.

The very marked loss in body weight produced by the higher doses of the estrogens is well known (cf. 8). It resembles the results of inanition. The amount of food eaten, however, was not determined.

It is difficult to state whether the toxicity of the estrogens was due to the effects of urinary retention or estrogen imposed starvation. It is of special significance that testosterone instead of protecting against these phenomena actually exacerbated them. The estrogens, on the other hand, had a marked inhibitory effect on the androgenic property of testosterone. Numerous reports (cf. 8) indicate that androgens and estrogens may have a mutually antagonistic or coöperative effect on various parts of the genital tract depending on relative doses.

The decrease in the androgenic activity of testosterone by MDDA and  $\alpha$ -estradiol was not accompanied by a similar decrease in renotrophic activity. Thus,

<sup>4</sup> Katherine Clancy and Carolyn Abbott carried out these determinations.

the combination of the two hormones produced a relationship between the weights of these two organs similar to that produced by androstanediol-3 $\alpha$ , 17 $\alpha$  and its 17-methyl derivative (1, 7, 9).

The smaller increase in the arginase activity of the kidney at the higher than at the lower dose of  $\alpha$ -estradiol and MDDA was probably due to the greater toxicity and loss of body weight. Inanition decreases the kidney arginase-stimulating property of the androgens (4). It is noteworthy that there was a summation of the arginase stimulating property of testosterone and estrogens. This is in contrast to the effect of the anterior pituitary growth hormone which markedly decreases this property of testosterone without affecting the androgen's renotrophic property (unpublished).

The failure of the estrogens to produce any changes in the kidney phosphatases is in agreement with the previous study (2). It is of special significance that the urinary retention was not accompanied by a decrease in the 'alkaline' phosphatase, since this is the first change noted after ligation of the ureters (10). Indeed this enzyme and also arginase nearly completely disappear as the kidneys become non-functional (11).

The remarkable increase in the arginase activity of the liver produced by MDDA is specially noteworthy. This phenomenon was not apparent, moreover, until a very great excess of the estrogen was absorbed—a reverse of the enzyme effect noted in the kidney. The effect of MDDA on the 'alkaline' phosphatase of the liver was even greater than that on the arginase. Furthermore, this increase was noted at the lower dose and also after  $\alpha$ -estradiol treatment. A smaller increase in the 'acid' phosphatase was noted only with the high dose of MDDA. The loss in body and liver weight suggests that the increases in these hydrolytic enzymes of the liver may be a reflection of the great demands placed on endogenous protein. Indeed, this may be indicative of the depletion of protein reserves and a breakdown of vital protein structures. The effect on the arginase and 'acid' phosphatase activities was not apparent until the dose of the estrogen was so great that it caused the death of many of the mice.

#### SUMMARY

Male mice castrated at 17- to 19-gm. body weight were implanted subcutaneously with a pellet of testosterone,  $\alpha$ -estradiol, methoxybisdehydroisynolic acid (MDDA), 1-methylestradiol and 1-methylestrone. The first two estrogens also were implanted as pellets consisting of one part of estrogen and three parts of cholesterol and simultaneously with a pellet of testosterone. All of the experiments were for 16 days. In addition the testosterone and  $\alpha$ -estradiol experiment was performed at 10 and 30 days. The rate of absorption of MDDA was about eight times and that of 1-methylestradiol 4 times that of  $\alpha$ -estradiol. The introduction of the 1-methyl group into estrone did not alter its rate of absorption from a pellet. The simultaneous implantation of a pellet of testosterone did not influence the rate of absorption of  $\alpha$ -estradiol or MDDA.

MDDA and  $\alpha$ -estradiol as pure pellets greatly reduced the body weight in a manner resembling inanition. There was a concomitant retention of urine which was

exacerbated by the simultaneous administration of testosterone. Also deaths occurred only in those mice that were implanted with both testosterone and pure pellets of MDDA or  $\alpha$ -estradiol.

The renotropic property of testosterone was not altered, but the androgenic property was greatly reduced by both  $\alpha$ -estradiol and MDDA. The increase in arginase activity produced by testosterone and MDDA or  $\alpha$ -estradiol summated when the androgen and either of the estrogens were administered simultaneously. The estrogens did not influence the effect of the androgen on either 'alkaline' or 'acid' phosphatase. MDDA at the high dose produced a remarkable increase in the arginase activity of the liver;  $\alpha$ -estradiol was ineffective. MDDA also produced a remarkable increase in the 'alkaline' phosphatase at the high dose and  $\alpha$ -estradiol a moderate increase at its high dose. MDDA stimulated a small increase in 'acid' phosphatase at the high dose;  $\alpha$ -estradiol was ineffective. 1-methylestradiol and 1-methylestrone were ineffective in all of the above tests.

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# EFFECT OF TESTOSTERONE PROPIONATE ON NITROGEN AND CHLORIDE EXCRETION AND BODY WEIGHT OF CASTRATED RATS DURING RECOVERY FROM FASTING<sup>1, 2, 3</sup>

CHARLES D. KOCHAKIAN, LAURA COHN, ELLEN QUIGLEY AND  
EDITH TRYBALSKI

from the Department of Physiology and Vital Economics, University of Rochester School of  
Medicine and Dentistry

ROCHESTER, NEW YORK

SINCE testosterone propionate is known to stimulate protein-anabolic processes (see 1-3 for reviews of literature), it was decided to determine whether this steroid would hasten protein repletion after exhaustion of reserve stores complete removal of food from adult rats until shortly before death.

## EXPERIMENTAL

*Animals.* The rats were of the Wistar strain from our colony.

*Diet.* Each rat received 9.5 gm. of the following diet: casein 16.7, sucrose 61.2, hydrogenated vegetable oil 7.4, celluloflour 1.8, Wesson's (4) salt mixture 3.7 and dry brewers' yeast (Fleischmann Co. 2019) 9.2. The ingredients were mixed in a Hobart mixer and kept at 5°C. Each new batch was analyzed for nitrogen. The average nitrogen content was 2.95 per cent. Supplements of two drops of cod liver oil and one drop of wheat germ oil<sup>4</sup> diluted 10-fold with wesson oil were given each day. Each rat ate all of its food except a small amount of spill, 0.1 to 0.3 gm/day, which was weighed and subtracted from the given amount. Distilled water was provided *ad libitum*.

*Procedure.* The rats were placed in individual metabolism cages similar to those used by Swift (5), figure 2, or metal cages in racks manufactured by the Norwich Wire Works (fig. 3). The temperature of the room was maintained at 78 to 80°F.

The rats were weighed and fed at the same time each day. The injections were made just before or after feeding. In the first experiment (fig. 2), fecal periods of seven days were separated by mixing 0.4 gm. of animal charcoal into the food of the last day of the period. The feces were collected daily and saved until the end of the period in 75 ml. of 30 per cent sulfuric acid in which they disintegrated on warming. Fecal nitrogen was not determined in the second experiment. The line of each rat was washed from the receptacle and the cage with warm distilled water. Thymol 3 per cent benzoic acid in 50 per cent ethyl alcohol (6) was used as a preservative. The urine collection periods were two, two and three days each week except in the prefasting period and the last days of *experiment 1* (fig. 2) when the collection periods were extended to seven days.

*Nitrogen determination.* The micro-Kjeldahl procedure was used for all nitrogen determina-

Received for publication September 22, 1948.

<sup>1</sup> This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

<sup>2</sup> Mr. James McWhirter assisted in the care and feeding of the rats in the first experiment and an Moe carried out the fecal nitrogen determinations.

<sup>3</sup> The data contained in this paper have been reported in part in the transactions of the Josiah Macy Jr. Foundation Conference on Metabolic Aspects of Convalescence, eighth meeting, 58, 944 and tenth meeting, 79 (1945).

<sup>4</sup> The wheat germ oil was provided by Distillation Products Inc. through the courtesy of Dr. L. Harris.

tions except that aliquots of the diets were digested by the macro procedure, made to a given volume and duplicate aliquots distilled by the microprocedure.

*Chloride determination.* The method of McKittrick and Schmidt (7) was used for the determination of the chloride in the urine and the diet of *experiment 1*.

## RESULTS

*Determination of length of survival of rats without food.* In order to determine how long the rats of our colony could survive without food, 5 adult male rats, varying in body weight from 250 to 348 gm. (fig. 1), from the stock colony were placed in individual cages without food but with tap water *ad libitum* until they died. The body weights were recorded daily. The average survival period was  $15.4 \pm 1.7$  days. The body weight decreased to  $45.6 \pm 4.1$  per cent of the original body weight.

*Experiment 1. Prolonged injections of testosterone propionate.* The rats were castrated approximately 150 days before the beginning of the fasting period. After 12 days without food one rat died. Death in all probability was due to starvation

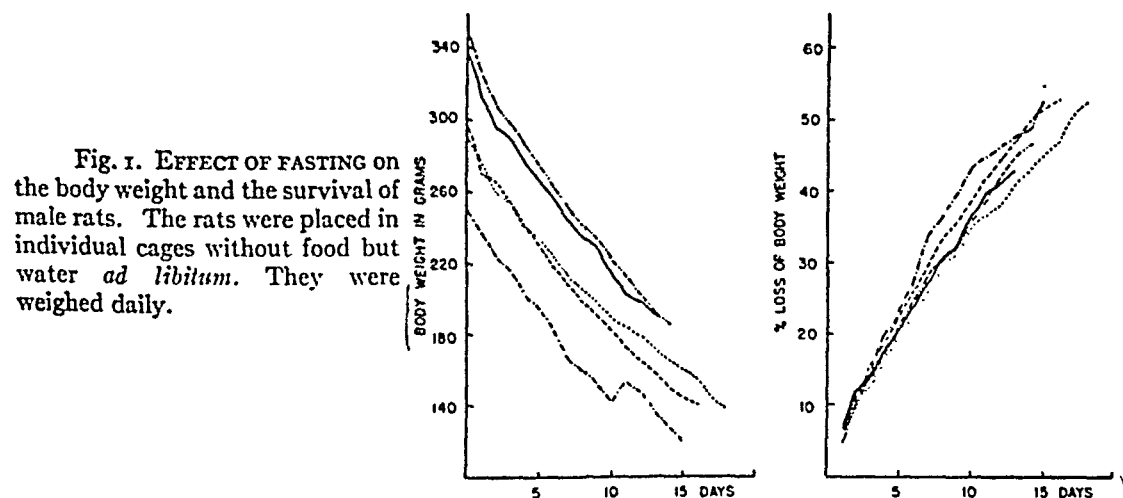


Fig. 1. EFFECT OF FASTING ON the body weight and the survival of male rats. The rats were placed in individual cages without food but water *ad libitum*. They were weighed daily.

for no gross pathology was observed. The survivors, therefore, were given food. At the same time 5 of the rats were injected with 2.5 mg/day of testosterone propionate<sup>5</sup> and the other 4 were injected with 0.1 ml/day of sesame oil.

It seemed at first that the rats injected with testosterone propionate were going to show a much greater increase in body weight than the controls (fig. 2). The initial spurt, however, was of relatively short duration, a maximum difference in body weight of 11 gm. was attained at the 20th and 21st days. It was followed by a much slower rate of gain in body weight so that by the 31st day the control rats had attained the same weight as the injected animals and continued to gain while the treated rats were no longer or only slightly increasing their body weight.

The rats which were to be injected lost an average of 75.4 gm. of their body weight during the period of fasting and the control rats 79.0 gm. The average loss of the two groups was 77 gm. At the end of the experiment the former animals had

<sup>5</sup> The testosterone propionate was provided as perandren by Ciba Pharmaceutical Products, Inc.

regained 68.4 gm. and the latter, 76.8 gm. of their body weight. The increase in seminal vesicles and prostates contributed 5.7 gm. to the body weight of the injected rats. Therefore, the control rats gained 8.4 gm. more than the injected rats, but if the accessory sex organs were discounted they gained 14.1 gm.

The testosterone propionate stimulated an extra nitrogen retention of 0.68g gm. or the equivalent (8) of 20.7 gm. of tissue. Practically all of this retention

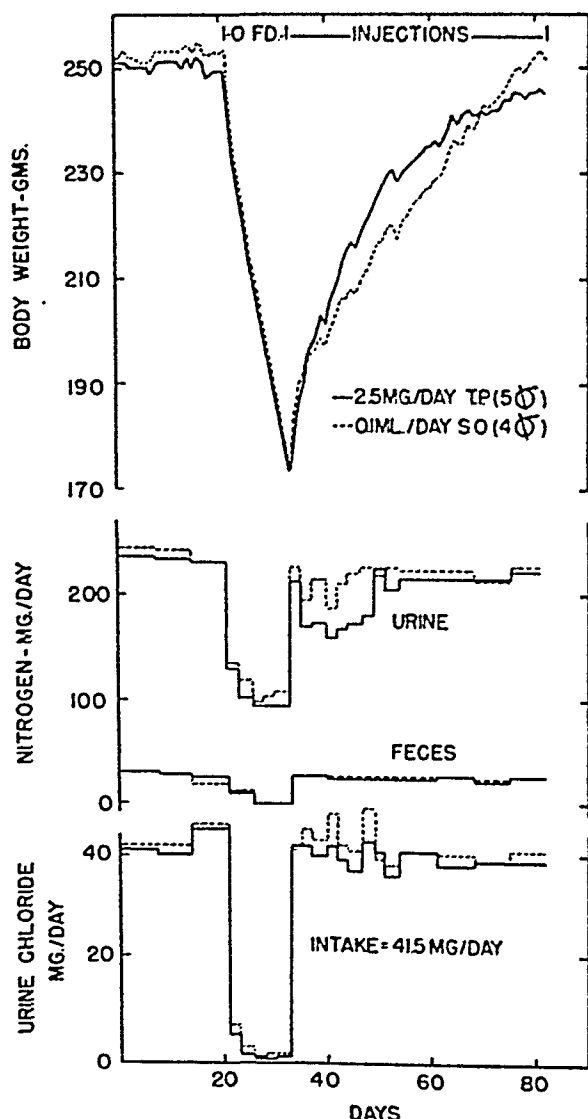


Fig. 2. *Experiment 1. EFFECT OF TESTOSTERONE PROPIONATE on the recovery of castrated male rats from a 12-day fast. The rats were fed a constant amount of food before and after the fasting period. T.P. = testosterone propionate; S.O. = sesame oil.*

occurred during the first 16 days of rehabilitation and accompanied the initial spurt of increase in body weight. Thereafter, the two groups of rats excreted approximately the same amount of nitrogen. At no time, however, did the injected animals lose any of the retained nitrogen. The nitrogen retained was due entirely to excretion of less nitrogen in the urine. The fecal nitrogen was not affected by the injections of the androgen.

The testosterone propionate injected rats excreted a consistently smaller amount

of chloride than the control rats during the periods of nitrogen retention. It is of interest that the amount of chloride excreted in the prefasting and the post-retention periods was identical to that fed. Also that the amount in the urine rapidly disappeared on the withdrawal of food and immediately returned to its previous level on refeeding.

*Experiment 2. Post-injection effects.* The rats had been castrated and their food intake adjusted over a period of 150 days so that they were in body weight equilibrium for several weeks prior to starvation. The results during the fasting and the injection periods were remarkably similar to those obtained in the previous experiment. Thus a decrease in dose from 2.5 to 1.0 mg/day did not lower the effectiveness of the androgen.

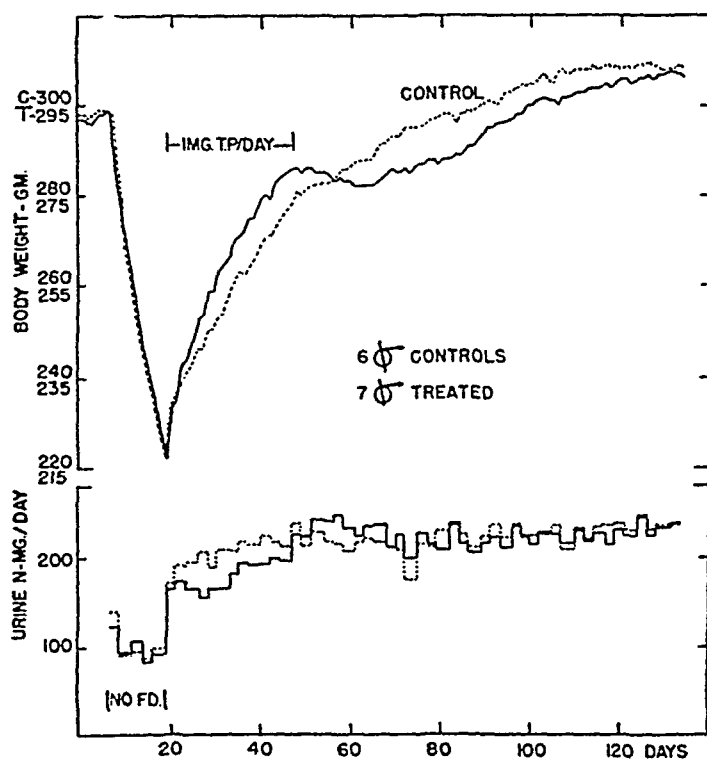


Fig. 3. *Experiment 2.* EFFECT OF TESTOSTERONE PROPIONATE and subsequent withdrawal on the recovery of castrated male rats from a 12-day fast. The rats were fed a constant food intake before and after the fast.

Both groups of rats lost exactly the same amount, 76 gm., in body weight during the 12 days of starvation. The testosterone propionate accelerated the restoration of the body weight so that during the 17th to 20th days the treated rats had gained 10 gm. more than the controls. Thereafter, the difference in body weights began to decrease and on the 28th day the injections were stopped. An immediate cessation followed by a small loss, 4 gm., in body weight resulted. Part of this loss in weight was undoubtedly due to regression of the accessory sex organs (cf. 9-11). The control rats, on the other hand, continued to increase in weight and soon surpassed the treated rats. After the initial depression in body weight, the treated rats began to increase in weight again and at a faster rate than the controls. At about the 130th day the two groups of rats weighed about the same and their body weights were progressing at the same rate.

The changes in urinary nitrogen excretion paralleled the changes in body weight. The control rats excreted an insignificantly greater amount, 32 mg., of nitrogen during the 12-day starvation period. The average values for the last 10 days, however, were identical for both groups. The injection of the androgen markedly increased the retention of nitrogen. The greater retention of nitrogen was immediately apparent and continued at the same rate for 14 days, then began to decrease, but was still apparent at the termination of the injections. The androgen stimulated a total nitrogen retention of 630 mg., or the equivalent (8) of 18.4 gm. of tissue, more than the controls during the 28 days of injection. On cessation of injections, the treated rats lost 399 mg. of nitrogen, equivalent (8) to 11.7 gm. of tissue, during the first 35 post-injection days but retained 134 mg., or the equivalent (8) of 3.9 gm. tissue, during the subsequent 16 days. Thus, the injected rats had an overall positive nitrogen balance of 365 mg. or 10.7 gm. of fat-free protoplasm more than the control rats. Since the body weights of the two groups of rats were restored to the same level, the injected rats probably lost an equal amount of fat (12).

#### DISCUSSION

These experiments demonstrate that testosterone propionate not only accelerates but also increases the degree of protein repletion after exhaustion of reserve stores by starvation. The effect, however, soon 'wears off' in spite of the fact that the animals are in a state of repletion. It is not due to the inhibition of growth stimulators from other endocrine organs. The same phenomenon has been observed in normal, castrated, hypophysectomized and adrenalectomized male rats and in normal and ovariectomized female rats injected with testosterone propionate while on a constant food intake (12).

The cessation of nitrogen retention, however, does not mean that the androgen is no longer effective. Metabolic processes still are stimulated. The accessory sex organs and the kidney continue to grow (12). Since there is no further nitrogen retained, the essential materials for these organs must be obtained directly or diverted from other tissues, e.g. muscle. This preferential stimulation to growth of the accessory sex organs at the expense of other tissues also has been observed in chronically undernourished mice (13) and in fasting dogs (14).

The changes in body weight are similar to those observed in growing young adult normal and castrated rats injected with testosterone propionate (12).

The differences between the observed changes in body weight and those calculated from the nitrogen retained probably are due to a smaller deposition and/or a greater utilization of fat. The chloride values of the urine indicate that no excessive gains or losses in water occurred. Furthermore, testosterone propionate decreases the fat content of the carcass of rats fed either a constant amount or *ad libitum* (12) and mice fed *ad libitum* (12, 15).

The extra nitrogen retained by the androgen-treated rats was much more than could be accounted for by the increase in weight of the seminal vesicles and prostates. In the first experiment, nitrogen equivalent to 20.7 gm. of tissue was retained while the seminal vesicles and prostates weighed 5.7 gm. In the second experiment, there was a loss of much of the retained nitrogen after cessation of injections, but the

equivalent of 10.7 gm. of tissue still was retained at the end of the experiment. This amount of protoplasm was incorporated into tissues other than the accessory sex organs, kidney or liver for these organs rapidly regress on withdrawal of androgen (9-11).

It may be concluded that testosterone propionate accelerates protein replenishment, but the treatment should be confined to the period of nitrogen retention. The androgen has been used successfully in several clinical studies (cf. 1-3).

#### SUMMARY

Adult white castrated rats were brought into body weight equilibrium and about 150 days after castration were subjected to 12 days of starvation. They were then fed a fixed amount of food and one-half of the animals were injected with testosterone propionate at 2.5 mg/day in the first and 1.0 mg/day in the second experiment. The rats injected with the androgen showed an initially more rapid rate of gain in body weight accompanied by a greater retention of nitrogen. After about the 20th day the body weight began to increase at a much slower rate so that if the injections were continued the weight of the control rats attained and surpassed that of the injected rats. At the same time the nitrogen retention decreased so that there was no difference between the amount of nitrogen excreted by the control or injected rats. On the other hand, if the injections were stopped after 28 days, there was at first a cessation and a loss in body weight accompanied by a loss in nitrogen, then the body weight increased to that of the controls. The nitrogen retained was much more than could be accounted for by the increase in size of the seminal vesicles and prostates. Furthermore, there was a loss of other material, probably fat, for the observed body weight changes were less than calculated from the nitrogen retained. The fecal nitrogen and urinary chloride excretions were not significantly affected by the androgen.

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# RENAL CLEARANCE OF ALLANTOIN AS A MEASURE OF GLOMERULAR FILTRATION RATE<sup>1</sup>

MEYER FRIEDMAN, SANFORD O. BYERS AND PAUL M. ABRAHM

*Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital*

SAN FRANCISCO, CALIFORNIA

IN A previous study (1) the renal clearance of allantoin in the rat and dog was found to be similar to that of exogenous creatinine. Furthermore, the allantoin clearance of the dog was found to be independent of the blood concentration of allantoin. These two observations of course suggested that the renal clearance of allantoin was a measure of glomerular filtration rate in these two species. This last phenomenon was not surprising after the physico-chemical similarity of allantoin and creatinine was discerned.

In view of the fact that the renal tubule of the rat and dog seemingly could not reabsorb allantoin (a substance naturally present in their blood) from the glomerular filtrate, it seemed possible that the renal tubule of the human subject (whose blood usually contains no allantoin) also might not be able to reabsorb allantoin. Accordingly, the renal clearance of allantoin was measured in a series of normal subjects and in patients suffering from cardio-renal disorders. The clearances obtained also were compared with those of inulin when the latter clearances were done concomitantly in the same subjects. The results of these clearances suggested that the clearance of allantoin in man was a measure of the glomerular filtration rate.

## METHODS

*Performance of the Clearances.* Preliminary studies (2) had indicated that an average blood concentration of approximately 5 to 6 mg. of allantoin per 100 cc. was obtained two hours after the oral ingestion of 10 gm. of allantoin. Furthermore little change occurred in the blood concentration three hours after oral ingestion. Accordingly, each patient received 10 gm. of allantoin dissolved in 500 cc. of orange juice at 8:00 A.M. on the day of the experiment. He was then kept at bed rest until 9:30 A.M. at which time he was catheterized and given 1000 cc. of H<sub>2</sub>O by mouth. At 10:00 A.M. the bladder was emptied, washed out with normal saline solution, a blood sample taken and the first urine collection begun. At 10:30 A.M. the bladder was emptied again, washed out with saline solution and a second blood sample was obtained. The second urine collection began immediately after the bladder had been washed and emptied. At 11:00 A.M. the second and final collection period was terminated and a third blood sample was obtained. The allantoin clearance of each subject then was calculated as an average of the clearances obtained for the two collection periods and corrected to 1.73 square meters of surface area.

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Received for publication September 6, 1948.

<sup>1</sup> Aided by grants from the United States Public Health Service and the Wine Advisory Board of the State of California.

Subjects upon whom the inulin clearance also was performed were prepared for their allantoin clearance exactly as described above except that at 9:30 A.M. they received 10 gm. of inulin dissolved in 100 cc. of normal saline solution by vein in five minutes, followed by a continuous intravenous infusion of an inulin solution (200 mg. of inulin/100 cc. of normal saline solution) at a rate of 4 cc./minute until 11:00 A.M. The three blood and two urine samples obtained during the clearance periods were analyzed for both allantoin and inulin.

*Chemical Determination of Allantoin and Inulin.* Allantoin in blood plasma and urine was determined primarily according to the method of Christman *et al.* (3) with an adoption of one of the suggestions of Young and Conway (4).

The actual procedure for small quantities of plasma was as follows: plasma samples were prevented from clotting by the addition of lithium oxalate (1.5 mg/cc. of original blood). Six volumes of a washed yeast-saline suspension (3 gm. of wet washed yeast in 12 cc. of normal saline solution) were added to one volume of plasma, mixed and allowed to ferment for 10 minutes at room temperature with occasional shaking. Plasma proteins then were removed with one volume of 10 per cent sodium tungstate solution and two volumes of N/3 sulphuric acid. Two cc. of protein-free supernatant fluid were pipetted into a test tube, the remainder being reserved for uric acid assay. Equal volumes also of 1) a reagent blank (distilled H<sub>2</sub>O), 2) a uric acid correction blank (0.5 mg. % uric acid), 3) a standard solution of allantoin (0.2 mg. % allantoin) and 4) a yeast blank (protein-free supernatant fluid from yeast-saline suspension) were made up at this point and placed into similar test tubes.

To each tube, 0.4 cc. of a N/2 NaOH was added, the contents mixed and the tubes placed in a boiling water bath for seven minutes. They were cooled for three minutes in a bath at 20°C. The N/2 HCl (0.52 cc.) then was added to each tube and mixed and the tubes were immersed in boiling water for exactly two minutes. They were immediately transferred and cooled in a bath at -10°C. for three minutes. Ice-cold phenylhydrazine (0.4 cc. of a 0.33 % solution of phenylhydrazine hydrochloride) was added to each tube and mixed and the reaction allowed to proceed for 15 minutes in a bath maintained at 30°C. After this, the tubes were immersed again in a bath at -10°C. for three minutes. At the end of this time, 1.6 cc. of 10 N/HCl (previously cooled to -10°C.) were added to each tube. Then, without mixing, 0.4 cc. of a potassium ferricyanide solution (1.64 gm. % ) previously cooled to 0°C. was added to each tube. A clock was started, each tube mixed by inversion against waxed paper and the contents of each tube poured into respective colorimeter tubes placed and kept in a water bath at 20°C. The color density was read in a Klett colorimeter against green filter No. 54. The entire series of tubes were read in succession, then readings were repeated until at least three had been obtained for the entire series between 6 and 30 minutes after color development had started. Calculations were made from the maximum values recorded.

A separate assay for the uric acid content of each unknown was performed according to the method of Folin (5), in order to determine the correction value.

The usual correction for uric acid was found to be 20 per cent of the value obtained for an equal concentration of allantoin. It should be stressed that for clin-



ical purposes a standard correction value may be assured without individual determinations of plasma uric acid, if the latter is not pathologically elevated.

Determination of the allantoin content of urine samples was carried out exactly as above except that 1) the urine was usually diluted until its allantoin content was

TABLE 1. RENAL CLEARANCE OF ALLANTOIN AND INULIN IN NORMAL SUBJECTS AND IN PATIENTS WITH CARDIORENAL DISORDERS

PT.	SEX	AGE	DISORDER PRESENT	U.V. <sup>1</sup>	P.A.C. <sup>2</sup>			A.C. <sup>3</sup>	I.C. <sup>4</sup>	A.C./I.C. <sup>5</sup>
					10:00	10:30	11:00			
A. Normal subjects										
WJ	M.	45	None	13.5	7.4	7.0	7.5	107.0		
HB	M.	22	"	3.7	6.1	7.1	6.8	130.0		
GC	F.	43	"	10.0	5.5	5.1	4.3	137.0		
CR	M.	25	"	11.0	4.8	4.8	6.1	112.0		
GB	M.	25	"	7.5	3.3	4.3	4.1	147.0		
HS	M.	43	"	5.7	6.9	7.7	8.8	123.0	117.0	1.05
DC	M.	32	"	4.3	4.3	4.3	4.8	115.0	96.0	1.20
FT	M.	28	"	8.0	4.6	7.4	4.0	116.0	111.0	1.04
RO	M.	35	"	4.1	8.8	8.4	8.0	118.0	135.0	0.88
Average.....				7.5	5.7	6.2	6.0	123.0	115.0	1.04

*B. Patients*

RD	M.	31	Hypertension	1.8	9.2	11.0	8.4	96.0	98.0	0.98
JG	F.	36	"	3.6	6.8	7.4	7.3	119.0	118.0	1.01
DD	M.	53	"	4.1	6.7	7.4	7.1	98.0	104.0	0.94
RF	F.	51	"	5.5	8.3	8.0	8.1	104.0	101.0	1.03
MM	M.	52	"	8.4	8.6	10.8	11.5	113.0	111.0	1.02
AM	F.	24	"	8.3	5.9	6.2	5.5	92.0	104.0	0.88
KK	M.	53	"	3.6	6.9	6.8	6.9	85.0	89.0	0.96
AB	M.	37	"	4.5	4.2	4.5	3.5	105.0	123.0	0.85
RN	M.	14	Coarctation	4.1	6.9	7.4	7.3	84.0	81.0	1.03
ND	M.	53	Nephritis	4.4	6.8	7.0	7.1	97.0	103.0	0.94
VN	F.	36	"	3.5	6.8	7.1	7.1	57.0	45.0	1.26
Average.....				4.7	7.0	7.6	7.3	95.0	98.0	0.99

<sup>1</sup> U.V. equals urine volume in cc/min. <sup>2</sup> P.A.C. equals plasma allantoin concentration in mg/100 cc. <sup>3</sup> A.C. equals allantoin clearance in cc/min./1.73 sq. M. <sup>4</sup> I.C. equals inulin clearance in cc/min./1.73 sq. M. <sup>5</sup> A.C./I.C. equals ratio of allantoin to inulin clearance in cc/min.

in the range of the standard allantoin solution and 2) preliminary treatment with yeast and deproteinizing chemicals was omitted.

Inulin in plasma and urine was determined according to the method of Hubbard and Loomis (6) modified, however, in that the hydrolysis of inulin was accomplished by the addition of a mixture of two parts of saturated alcoholic HCl and one part of concentrated aqueous HCl alone. Occasionally, turbidity may result following the addition of this mixture to blood filtrates, but this disappears on heating. The

hydrolysis itself was carried out for 10 minutes at 78°C. The resultant color development was read in a Klett photoelectric colorimeter with filter No. 54.

### RESULTS

As table 1 *A* indicates, the average allantoin clearance of 9 normal subjects was 123.0 cc/minute (range: 107.0–147.0 cc/min.). The average inulin clearance performed on 4 of these subjects was 115 cc. (range: 96.0–135 cc.). The ratio of the allantoin to the inulin clearance in these 5 subjects was 1.04 (range: 0.88–1.20). Most of the 11 patients studied (table 1 *B*) were suffering from hypertension of the malignant type. Thus the average allantoin clearance was reduced, being 95.0 cc/minute (range: 57.0–119.0 cc.). The average inulin clearance also performed upon these subjects was 98.0 cc/minute (range: 45–123.0 cc.). The ratio of the allantoin to the inulin clearance was 0.99.

### DISCUSSION

The similarity in values given by the allantoin and inulin clearances, when performed concomitantly upon both healthy subjects and upon patients suffering from possible reduced renal function, left little doubt that the former type of clearance was a measure of glomerular filtration rate in man just as it had been found to be in the rat and dog (1).

Besides the absence of signs of toxicity following the ingestion of allantoin and the relatively simple and straightforward chemical determination of its presence in blood and urine, its utilization in the estimation of a subject's renal filtration rate offered another advantage. Thus, the solubility of allantoin and its ability to be absorbed from the gastrointestinal tract at an apparently stable rate allowed the opportunity to perform a clearance without the necessity of a continuous intravenous infusion of the substance in a large volume of fluid during the test itself. Furthermore, catheterization is not mandatory since the patient may rise to void his urine during the clearance test.

### SUMMARY

The average renal allantoin clearance of normal human subjects was found to be 123.0 cc/minute. When both the allantoin and inulin clearances were performed on the same subject, they were approximately the same. It appears that the renal clearance of allantoin offers a very safe and simple assessment of the glomerular filtration rate in both the normal subject and one suffering from cardiorenal disease.

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# SIMULTANEOUS CLEARANCE DETERMINATIONS IN THE RAT

RICHARD W. LIPPMAN<sup>1, 2</sup>

*From the Department of Medicine, Stanford University School of Medicine*

SAN FRANCISCO, CALIFORNIA

*and the Institute for Medical Research, Cedars of Lebanon Hospital*

LOS ANGELES, CALIFORNIA

**S**IMULTANEOUS measurement of glomerular filtration rate by inulin clearance, and of renal plasma flow by p-aminohippurate (PAH) clearance, has become a widely used and accepted practice. If such determinations are to have more than relative validity, it is necessary to know that the clearance substances, in required quantities, do not mutually affect the values which they are intended to measure. This study was performed to investigate such interrelationships in the rat.

## METHODS

Clearances were measured by the 'tailcutting' method previously reported in detail (1). The clearance substance was injected subcutaneously and blood was obtained from the tail at the start of a clearance period. Urine was collected for one hour and blood was obtained from the abdominal aorta at the end of the clearance period.

The use of groups has been discussed in our earlier reports. In this study 253 normal rats were used, divided into groups of 8 to 10 rats each. The groups were chosen for uniformity in size and sex and specimens of blood and urine within each group were pooled for determination.

Inulin was determined by the method of Alving, Rubin and Miller (2). The PAH was determined by the method of Smith *et. al.* (3).<sup>3</sup> In calculating clearances, mean serum concentrations were used, obtained by assuming a logarithmic rate of fall from the initial to the final concentration, after a single injection. Kidney weight (KW) was measured at autopsy after the clearance period and the clearance was expressed in terms of the measured weight.

## RESULTS AND DISCUSSION

The inulin clearance fell steadily as the serum concentration of PAH was increased. At a PAH level of 62.5 mg/100 cc., the inulin clearance was about 65 per

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Received for publication September 16, 1948.

<sup>1</sup> This work was aided by a grant from the Division of Research Grants and Fellowships, National Institute of Health, U. S. Public Health Service.

<sup>2</sup> The author, now at the Institute for Medical Research, Cedars of Lebanon Hospital, gratefully acknowledges the technical assistance of Evalyn Barrett and Way Lew of Stanford University, School of Medicine.

<sup>3</sup> Because the results differed so strikingly from those expected, it was felt necessary to verify the determinations. One group of pooled specimens from 18 rats was divided equally. We performed determinations on one part. Dr. Meyer Friedman and Dr. Sanford Byers analysed the other portion at the Harold Brunn Institute for Cardiovascular Research of the Mt. Zion Hospital, San Francisco. We are deeply grateful for the cooperation received, which enabled us to feel confident in our determinations, as good confirmation was obtained.

cent of that measured in the absence of PAH (table 1). The PAH clearance (table 2) fell substantially in the presence of increasing serum inulin concentrations. At

TABLE 1. RELATION OF INULIN CLEARANCE TO INCREASING SERUM PAH CONCENTRATION

GROUP NO.	MEAN GM. BW	MEAN MG. KW	URINE VOL. CC/MIN/GM. KW	MIDPOINT SERUM PAH CONC. MG/100 CC.	INULIN CLEARANCE CC/MIN/GM. KW
75A	181	1165	.004	0	1.13
75B	183	1204	.024	0	1.12
69	170	1108	.020	0	1.25
74	152	964	.008	0	1.16
77	161	1111	.014	2.76	1.19
78	144	992	.008	3.94	1.01
54	167	1051	.009	4.01	1.12
53	167	1131	.009	7.58	1.02
66	176	1164	.009	8.16	1.25
84	159	1135	.015	9.29	1.18
61	176	1092	.009	10.40	1.00
86	154	1109	.010	11.32	1.08
71	175	1190	.011	17.65	0.998
67	179	1172	.011	23.9	0.951
68	174	1157	.013	26.2	0.916
82	152	1040	.013	33.5	1.04
90	166	1146	.007	36.3	0.894
99A	173	1172	.014	38.1	0.916
58	184	1099	.013	38.2	0.863
101	176	1251	.012	38.6	0.873
76	160	1059	.013	46.5	0.837
143	155	1040	.013	49.1	0.788
81	158	1115	.014	62.5	0.759

TABLE 2. RELATION OF PAH CLEARANCE TO INCREASING SERUM INULIN CONCENTRATION

GROUP NO.	MEAN GM. BW	MEAN MG. KW	URINE VOL. CC/MIN/GM. KW	MIDPOINT SERUM INULIN CONC. MG/100 CC.	CLEARANCE PAH CC/MIN/GM. KW
31	176	1037	.024	0	4.02
30	179	1088	.020	0	3.90
28	181	1162	.016	0	4.67
25	181	1178	.011	0	3.90
23	181	1218	.008	0	4.35
84	159	1135	.015	30.9	3.34
77	161	1111	.014	46.8	3.73
54	167	1051	.009	59.6	3.05
61	176	1092	.009	60.0	2.66
66	176	1164	.009	60.0	3.18
86	154	1109	.010	64.9	3.01
53	167	1137	.009	70.8	3.28
78	144	992	.008	73.8	2.87

an inulin concentration of 73.8 mg/100 cc., the PAH clearance was about 69 per cent of that measured in the absence of inulin.

By plotting the excretion of PAH against the serum PAH concentration, in the presence of a relatively constant serum inulin concentration, a break in the graph was observed at a level of 15 to 20 mg/100 cc. (fig. 1). This presumably defines the level at which tubular saturation is obtained.

The possibility that simultaneously administered substances might interfere with the measurement of renal function has been suggested previously. Klopp, Young, and Taylor (4) found in man that high plasma glucose concentrations depressed PAH clearance and  $Tm_{PAH}$ , while high plasma PAH concentrations increased the tubular reabsorption of glucose. In the dog, Houck (5) found that  $Tm_{PAH}$  was depressed by high plasma glucose concentrations, but  $Tm_{glucose}$  was also depressed by high plasma PAH concentrations, though the effect was not as marked. Crawford (6) recently found that the ratio of exogenous creatinine clearance to inulin or

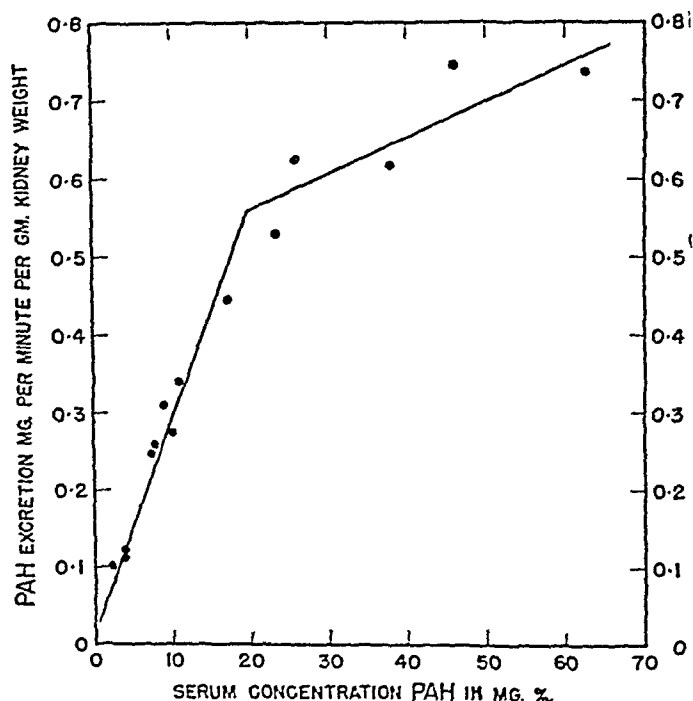


Fig. 1. RELATION OF PAH excretion to serum concentration.

thiosulfate clearances was reduced in man by saturation with diodrast or PAH. Previous reports of determinations on rats (7-11) have not considered the possible interference of clearance substances.

The findings just mentioned concern interferences between processes, both mediated by tubule cells: secretion and reabsorption. Our findings indicate that a substance administered to measure tubular function affects the measurement of glomerular filtration rate in the rat, as well.

The results presented here might be a consequence of several different factors: 1) pharmacological action of the substances used, 2) impurities in the chemical substances and 3) chemical interference in the methods of determination. We can dispose of the last possibility easily. Increasing amounts of PAH did not affect the determination of a standard inulin solution in concentrations simulating those obtained in the clearance studies. Likewise, increasing amounts of inulin did not affect the determination of a standard PAH solution.

It is well known that large doses of PAH produce transient vasomotor effects in man and it is not unreasonable to suppose that similar effects occur with more persistence in the rat. The PAH solution ordinarily obtained contains a brown discoloration, presumably due to oxidation products, offering another source of vasomotor disturbing agents.

We have consistently observed that, after injecting PAH for clearance determinations, the difficulty in obtaining tail blood specimens is directly proportional to the dose of PAH. In addition, after large doses of PAH, the superficial foot veins appear markedly collapsed. It may be noted in table 1 that the reduction in inulin clearance first becomes pronounced as PAH saturation levels are approached, while no effect is perceived at very low PAH concentrations.

Friedman, Polley and Friedman (11) state that tubular saturation for PAH is reached at plasma concentrations of 5 to 6 mg/100 cc. They found no maximum value for tubular excretion of PAH and suggested that the filterable portion of PAH changes at high concentrations.<sup>4</sup> It would seem from their data that none of their determinations were performed at levels sufficiently elevated to obtain tubular saturation.

From these observations, it is concluded that interference between inulin and PAH, as obtained commercially, prevents their simultaneous use in the rat to obtain physiological values for glomerular filtration rate and maximum rate of tubular excretion. Values obtained simultaneously will reflect variations in the concentration of the clearance substances, as well as the conditions of measurement and physiological changes.

#### SUMMARY

Increasing serum concentrations of PAH reduced the inulin clearance in the rat by one third, an effect that was perceived as the serum PAH concentration approached tubular saturation levels. Increasing serum concentrations of inulin reduced the PAH clearance similarly.

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<sup>4</sup> In a personal communication, Dr. E. Newman suggests that the degree of PAH acetylation may vary in the rat with serum concentration, as it does in man. However, in unpublished data, we have obtained reasonably constant values for TmpAH at serum concentrations higher than 15 mg/100 cc.

# HEPATIC AND PERIPHERAL REMOVAL RATES, IN THE DOG, FOR INTRAVENOUSLY INJECTED BROMSULPHALEIN

CLARENCE COHN, RACHMIEL LEVINE AND MURIEL KOLINSKY

*From the Department of Biochemistry and the Department of Metabolism and Endocrinology, Medical Research Institute, Michael Reese Hospital<sup>1</sup>*

CHICAGO, ILLINOIS

IN ORDER to measure hepatic blood flow or maximal hepatic transfer capacity quantitatively, the substance employed must be handled exclusively by the liver. Extra-portal organs should neither metabolize this substance nor remove it from the blood stream. Although there is little experimental evidence that bromsulphalein (BSP) is removed from blood solely by the liver, Bradley, Ingelfinger, and associates (1, 2) use this dye for estimating hepatic blood flow and Mason *et al.* (3, 4) employ BSP for measuring maximal hepatic transfer capacity ('Lm'). Both groups of workers base their results on the contention that extra-hepatic and extra-portal sites of BSP removal can be 'saturated' so that they no longer participate in clearing blood of the dye.

In a previous publication (5), we reported that bromsulphalein could not be used to estimate hepatic blood flow, since the dye is not solely disposed of by the liver and portal organs. This paper presents additional evidence that BSP as now used is unsuitable for measuring either hepatic blood flow or hepatic maximal transfer capacity.

## METHODS

Two types of experiments were employed.

a) In order to determine whether the peripheral tissues of the dog could be 'saturated' with respect to their ability to remove BSP, eviscerated-hepatectomized-nephrectomized dogs were given a priming dose of the dye<sup>2</sup> (5-7 mg/kg.) followed by a constant intravenous injection (0.1-0.7 mg/kg/min.) of the material for periods up to three hours. Serial serum BSP levels were determined and maintained above a concentration of 20 mg. per cent. Except for the operative manipulations, conditions were similar to those employed by Mason to determine 'Lm' in intact dogs. Apparent 'Lm' values were calculated according to this investigator (4). For our calculations, we assumed 5 per cent of the intact body weight to be plasma.

b) The simultaneous rates of disappearance from the blood of BSP and Evans Blue after a single intravenous injection into eviscerated-hepatectomized-nephrectomized dogs were followed for 60 minutes. These animals received 5 to 10 mg. of Evans Blue and 40 to 75 mg. of BSP immediately after evisceration. It was hoped

Received for publication September 14, 1948.

<sup>1</sup> These departments are in part supported by the Michael Reese Research Foundation.

<sup>2</sup> We are indebted to Hynson, Wescott and Dunning for the bromsulphalein used in these experiments.

that this type of experiment would aid in evaluating capillary permeability since increased permeability might account for the disappearance of BSP. Serum BSP concentrations and evisceration-hepatectomy-nephrectomy were performed as previously described (5). A correction was made in determining the BSP level for the absorption of light at 580 m $\mu$  due to Evans Blue.

TABLE 1. DISAPPEARANCE RATE ('PM') OF BSP IN EVISCERATED-HEPATECTOMIZED-NEPHRECTOMIZED DOGS RECEIVING A CONSTANT INTRAVENOUS INJECTION OF THE DYE

DOG	WEIGHT	TIME	TOTAL BSP INJ. DURING TIME PERIOD INDICATED	SERUM LEVEL	SERUM BSP		BSP DISAPPEARING	
					Total circulating	Circulating incr.		
	<i>kilo</i>	<i>min.</i>	<i>mg.</i>	<i>mg. %</i>	<i>mg.</i>	<i>mg.</i>	<i>mg/min.</i>	<i>mg/kg/min.</i>
1	10.0	0*						
		30	266	51.0	255			
		70	288	100.0	500	245	1.07	0.107
		100	216	128.0	640	140	2.53	0.253
		* 50 mg. of BSP as priming dose at time 0, followed by 7.2 mg/min. for duration of experiment						
2	7.5	0*						
		25	87.5	30.0	112.5			
		55	63.0	44.0	165	52.5	0.33	0.044
		82	57.7	55.0	206	41	0.62	0.082
		117	73.5	65.0	244	38	1.03	0.137
		147	63.0	73.0	274	30	1.1	0.147
		177	63.0	78.0	293	19	1.47	0.183
		* 35 mg. of BSP as priming dose at time 0, followed by 2.1 mg/min. for duration of experiment						
3	11.0	0*						
		27	84	18.8	103			
		62	44	21.2	117	14	0.86	0.078
		92	38	26.3	145	28	0.33	0.03
		122	38	28.8	158	13	0.83	0.076
		152	38	32.6	179	21	0.57	0.05
		182	38	35.0	193	14	0.80	0.073
		* 50 mg. of BSP as priming dose at time 0, followed by 1.26 mg/min. for duration of experiment						

#### RESULTS AND DISCUSSION

a) *Effect of a constant intravenous injection of BSP on total circulating quantity of dye in eviscerated-hepatectomized-nephrectomized dogs.* After a priming dose and constant intravenous injection of BSP, the results demonstrate a continual disappearance of some of the circulating dye. This should not have occurred had the peripheral depots been 'saturated'. Table 1 shows that when apparent 'Lm' values are calculated according to Mason, results are obtained which indicate that between 0.03 and 0.25 mg/kg/min. of dye disappear.

b) *Comparison of simultaneous rates of disappearance of BSP and Evans Blue*



after a single intravenous injection into eviscerated-hepatectomized-nephrectomized dogs. Bromsulphalein disappeared from serum more than three times as rapidly as Evans Blue which was injected at the same time into three dogs (fig. 1). Two of the three animals were followed with hematocrit values; these dogs showed a volume of packed red blood cells of 40 and 42 respectively immediately after evisceration. Sixty minutes later, the corresponding values were 39 and 42.

Our results indicate that bromsulphalein is removed from blood in the absence of the liver and portal organs. Although it is claimed that extra-hepatic and extra-

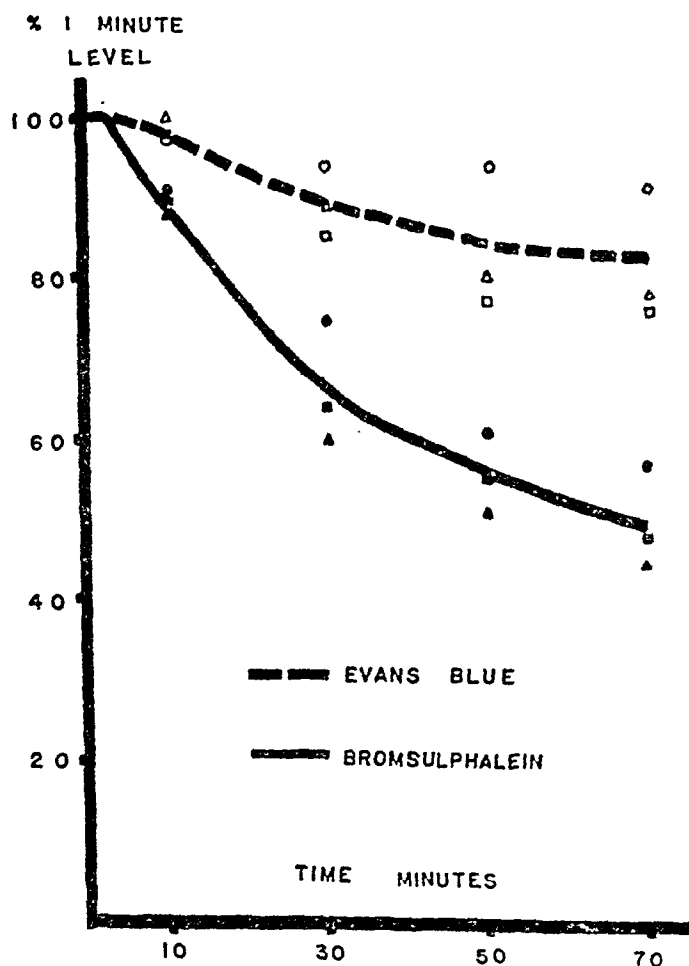


Fig. 1. SIMULTANEOUS RATES OF DISAPPEARANCE OF BSP AND EVANS BLUE after a single intravenous injection of both dyes at time 0 into eviscerated hepatectomized-nephrectomized dogs. The various points were determined by assigning the one-min. serum level a value of 100%.

Dog 1 (circles) 15 kg. Evans Blue = 10 mg. I.V. at time 0; one-min. serum level = 1.70 mg. %; BSP = 75 mg. I.V. at time 0; one-min. serum level = 11.0 mg. %.

Dog 2 (squares) 12.5 kg. Evans Blue = 7 mg. I.V. at time 0; one-min. serum level = 1.75 mg. %; BSP = 75 mg. I.V. at time 0; one-min. serum level = 15.25 mg. %.

Dog 3 (triangles) 8.5 kg. Evans Blue = 5 mg. I.V. at time 0; one-min. serum level = 1.75 mg. %; BSP = 40 mg. I.V. at time 0; one-min. serum level = 15.25 mg. %.

portal sites of BSP removal can be 'saturated' with the dye and therefore rendered incapable of extracting more of the material from blood, we have not been able to demonstrate this 'saturation' in the eviscerate preparation. In attempting to achieve 'saturation', we carried out our experiments with serum concentrations at least five times the 'saturating' level advocated (4). Mason believed that a serum BSP level of 4 mg. per cent was more than sufficient to 'saturate' extra-portal sites for the dye's removal, but we were unable to 'saturate' these sites with serum levels as high as 100 mg. per cent. On the contrary, our results actually give some indication that the percentage of BSP lost from serum at high concentrations is greater

than at low ones. This could be expected if an active peripheral removal mechanism was present. It thus becomes possible to calculate the 'Pm' (maximal peripheral transfer capacity for BSP). This capacity appears to vary from animal to animal and bears some relationship to serum concentrations of dye. Our results for 'Pm'—varying between 0.03 and 0.25 mg/kg/min.—are one-tenth to one-half of the values Mason reported for 'Lm' in intact animals. These results are based on calculations which assign 5 per cent of the body weight to plasma volume. Since the results on the Evans Blue experiments show the plasma volume of the eviscerate preparations to be closer to 4 per cent of the intact weight, actual 'Pm' values are even greater than calculated.

Evans Blue disappeared in the eviscerate-hepatectomized-nephrectomized preparation at an average rate of about 15 per cent in the first hour after injection. BSP, infused at the same time as the Evans Blue, was removed from plasma so rapidly that only 50 per cent remained 60 minutes after its injection. Since the reported values for the disappearance of Evans Blue in intact animals are given as between 3 and 12 per cent in the first hour after injection, (6–8), it becomes apparent that the disappearance of BSP cannot be explained on the basis of increased capillary permeability. Rather the results suggest active peripheral removal of injected bromsulphalein. Further proof of normal permeability is given by the constancy of the hematocrit values.

#### CONCLUSION

Bromsulphalein disappeared from the blood of eviscerated-hepatectomized-nephrectomized dogs more than three times as rapidly as Evans Blue, which was injected intravenously at the same time. No evidence for excessive loss of BSP through the capillaries could be demonstrated in the eviscerate preparation. It was possible to estimate 'Pm' (maximal transfer capacity of the periphery for bromsulphalein), since it was impossible to 'saturate' extra-hepatic and extra-portal sites of bromsulphalein removal with serum levels as high as 100 mg. per cent. The rate of removal of intravenously injected BSP, being the sum of both portal and peripheral removal mechanisms, invalidates the use of the dye for measuring either hepatic blood flow or of 'Lm'.

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# MECHANISMS OF DESOXYCORTICOSTERONE ACTION: IV. RELATIONSHIP OF FLUID INTAKE AND PRESSOR RE- SPONSES TO OUTPUT OF ANTIDIURETIC FACTOR

JULIA GOODSSELL SKAHEN AND D. M. GREEN

*From the Departments of Physiology, Anatomy and Medicine, University of Washington  
School of Medicine*

SEATTLE, WASHINGTON

**I**MPLANTATION of desoxycorticosterone acetate (DCA) pellets in rats is followed by a progressive elevation of fluid intake, complete in approximately 10 days (1, 2). The new intake level is related to that of controls of the same weight by a ratio fixed by dosage and degree of supplementary sodium chloride administration (2). Since fluid balance in the normal animal is presumed to represent an equilibrium between the adrenal cortex and the posterior pituitary (3), it was considered that the upward disturbance of this adjustment by an excess of desoxycorticosterone should evoke a counter-balancing output of antidiuretic factor.

The association between antidiuretic and pressor factors in posterior pituitary extracts suggested, furthermore, that the increase in blood pressure which occurs subsequent to DCA-induced elevation of fluid intake (4) might represent the pressor aspect of a combined posterior pituitary response. These possibilities were explored by comparison of the changes produced by DCA in fluid intake and blood pressure with the output of antidiuretic factor in the urine.

## PROCEDURE

The experimental animals for this study consisted of 40 immature rats of the Sprague-Dawley strain. All animals were kept in separate cages and were fed on Purina Laboratory Chow. Consumption of food and fluid was unrestricted. After a 2-week period of observation the animals were divided into two groups matched as to weight, sex and fluid intake. One group was given water as the drinking fluid while the other received isotonic (0.86%) salt solution.

Single 20 mg. DCA pellets with an absorption time of four to six months were implanted subcutaneously in 10 animals of each group, using ether anesthesia. The remaining animals were sham operated. Fluid intake was measured daily and weight weekly. Repeated blood pressure determinations were made by a modification of the tail method (5) commencing at the 12th week, the time at which maximum blood pressure elevation may be expected under the conditions stated (2).

Five weeks after pellet implantation, 48-hour urine collections were initiated and were repeated at approximately two-week intervals for five months. The collections were made under toluene, sufficient one per cent acetic acid being added to make the urine weakly acid. The first 24-hour sample was refrigerated until the

second day's collection was completed. A total of 30 collections from the salt-treated group and 10 from the water-treated group were assayed for antidiuretic activity.

*Preparation of Urines for Assay.* The procedure adopted for the preparation of the urine concentrates was similar to that used by previous investigators (6). The urines were dialyzed against running tap water until chloride free (5-6 hr.). The samples were then evaporated to dryness in shallow vessels at 34° to 38°C. Evaporation required approximately 48 hours, the time varying somewhat with the amount of urine. When amounts greater than 200 cc. were collected, aliquots were used.

On the day of assay the dried residues were dissolved in distilled water to form solutions from 4 to 10 times as concentrated as the original urine. These solutions were centrifuged and the supernatant solution decanted in order to obtain a clear fluid for injection. The  $pH$  was adjusted to lie between 7.5 and 8.5. No samples were stored, either wet or dry, for periods longer than five days.

#### METHOD

The rats used for assay of urinary antidiuretic factor output were adult males of the Sprague-Dawley strain, weighing 200 to 300 gm. The animals were maintained in Purina Fox Chow and were given water *ad libitum*.

The assay was performed by a modification of the method of Burn (7). Measurements of antidiuretic activity were based on the change in rate of renal excretion of water in hydrated rats after administration of the test substance. The activity of the urine concentrates tested was estimated by comparison with the delay in excretion produced by standard amounts of pituitrin in the same assay animals. Pituitrin (S), Parke, Davis and Company, containing 20 I.U./cc., was used as the reference standard and diluted with distilled water to final concentrations of 5, 10 or 15 mU/cc.

Precautions were taken to obtain as constant a response as possible. No groups were used for assay purposes more than once weekly. Animals which developed skin lesions at the injection site were discarded until healing had occurred. This complication developed more frequently in the animals injected with pituitrin than in those which received urinary concentrates or water. Whenever the total urine collection permitted, each concentrate was tested on two groups of animals and the results of multiple concentrations compared as an internal check on the reliability of the assay method.

On the day preceding assay all food was removed from the animals at 5 P. M. On the next day a preliminary dose of water amounting to 2½ per cent of body weight was administered orally three hours before assay time. At the time of assay the animals were hydrated by oral administration of water to the extent of 5 per cent of body weight. Immediately following hydration the urine concentrate to be assayed was injected subcutaneously in a volume amounting to 1 cc/100 gm. of body weight. The control animals were injected with an equal amount of water.

Following injection, the urine output was measured at 15-minute intervals. The period from the midpoint of the time taken to inject the assay group (av. time 6 min.), until the excretion of 50 per cent of the total amount of fluid administered at the time of assay, was used to calculate the antidiuretic activity of the urine concentrate under test.

## RESULTS

Analysis of a total of 90 control measurements of the normal 50 per cent excretion time and of the response to pituitrin indicated that the end point value was not sig-

TABLE 1. EFFECTS OF HYDRATION AND POSTERIOR PITUITARY EXTRACT ON URINARY OUTPUT.  
RELATIONSHIP TO WEIGHT OF ASSAY ANIMAL

PROCEDURE	WEIGHT RANGE		NO. OF ASSAYS	AV. 50% EXCRETION TIME	STANDARD DEVIATION	t VALUE (LOWER HALF VS. UPPER HALF OF WT. RANGE)
	<i>fraction</i>	<i>gm.</i>		<i>min.</i>	<i>min.</i>	
Hydration, 5% of Body Wt.	Lower 1/2	156 to 244	27	122	±12.6	0.66
	Upper 1/2	245 to 338	27	120	±10.6	
	Total	156 to 338	54	121	±11.6	
Hydration + Pituitrin 5 mU/100 gm.	Lower 1/2	195 to 246	8	172	±34.5	1.04
	Upper 1/2	247 to 276	8	156	±26.2	
	Total	195 to 276	16	164	±30.8	
Hydration + Pituitrin 10 mU/100 gm.	Lower 1/2	169 to 242	9	196	±25.6	-0.29
	Upper 1/2	243 to 326	9	200	±33.2	
	Total	169 to 326	18	198	±28.6	

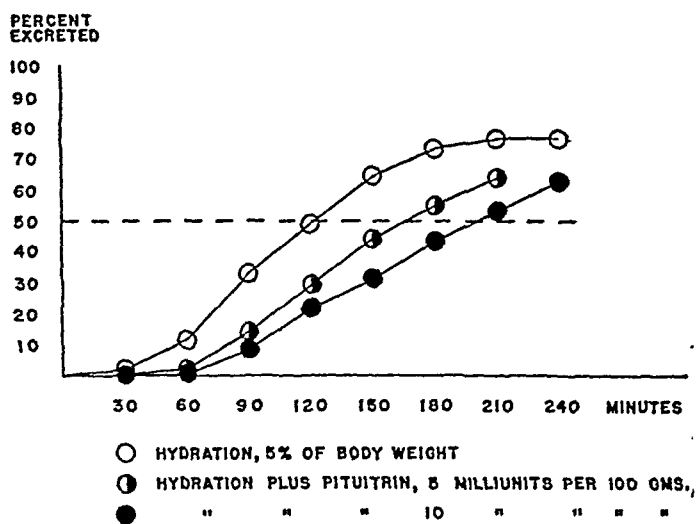


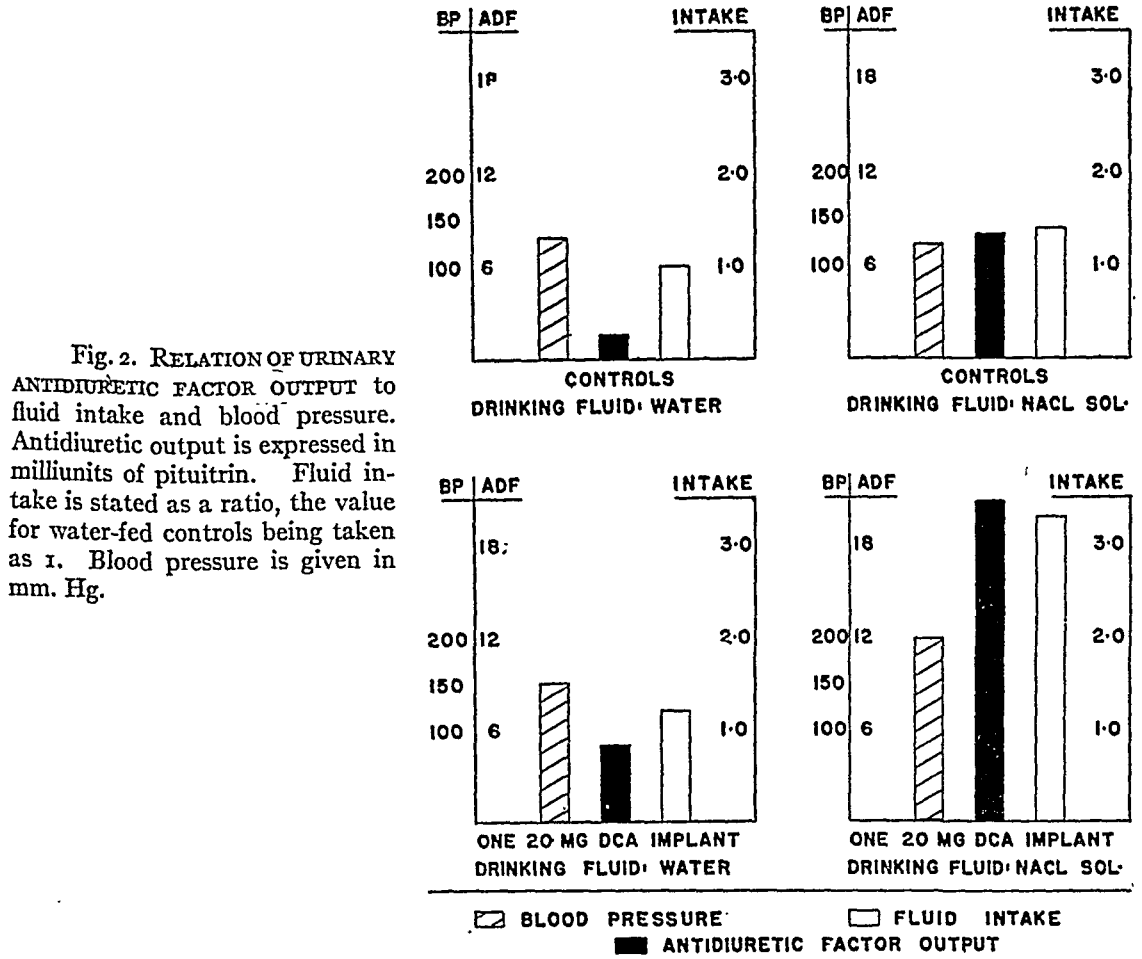
Fig. 1. URINARY EXCRETION RATES following hydration and pituitrin administration. Each composite curve is based on 10 representative assays.

nificantly influenced by the weight of the assay animals over the ranges studied (table 1). The average delay in excretion was directly proportional to the pituitrin dosage over a range of 0 to 10 mU/100 gm. of body weight (table 1, fig. 1). The

concentration of antidiuretic factor in the urine of the test animals was estimated on the basis of this relationship.

TABLE 2. ANTIDIURETIC FACTOR EXCRETION IN RATS FOLLOWING IMPLANTATION OF DCA PELLETS

GROUP	DRINKING FLUID	ANTIDIURETIC FACTOR EXCRETION MEAN AND S.D., MU. OF PITUITRIN	FLUID INTAKE (RATIO TO WATER-FED CONTROLS)	BLOOD PRESSURE, MEAN AND S.D., MM.HG.
Controls	Water	1.6 ± 2.4	1.00	131 ± 13
	NaCl 0.86%	8.0 ± 4.9	1.39	120 ± 8.6
One 20 mg. DCA implant	Water	5.0 ± 2.6	1.21	150 ± 21
	NaCl 0.86%	20.8 ± 8.7	3.31	196 ± 11



Inspection of the antidiuretic outputs of the various experimental groups (fig. 2) indicated a direct proportionality to fluid intake, not only in the implanted animals but in the saline-fed controls as well. The increase in these two values when saline administration was combined with desoxycorticosterone implantation was greater

than the sum of the increases produced by each procedure separately (table 2). The magnitude of the output, like the degree of intake elevation, was not shown to vary significantly throughout the observation period when once established.

In contrast, no immediate relationship between antidiuretic factor and blood pressure was evident. Water-fed animals which developed significant hypertension ( $t = 3.04$ ) as a consequence of desoxycorticosterone implantation excreted somewhat smaller amounts of the factor than did salt-treated controls in which blood pressures remained normal.

#### SUMMARY

Subcutaneous implantation of desoxycorticosterone in rats was followed by increases in fluid intake, blood pressure and urinary output of antidiuretic factor. Substitution of isotonic salt solution for drinking water in animals not treated with desoxycorticosterone produced an increased output of antidiuretic factor unaccompanied by significant blood pressure elevation. The urinary output of antidiuretic factor was proportional to the degree of elevation of fluid intake, whether increased intake was produced by salt administration alone, desoxycorticosterone alone, or a combination of both. The evocation of antidiuretic factor excretion by desoxycorticosterone would appear to be a consequence of the disturbance in fluid exchange. A direct relationship of this factor to the development of hypertension was not established.

We are indebted to Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, N. J. both for generosity in supplying desoxycorticosterone and for many helpful suggestions and criticisms throughout these studies.

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# WATER AND ELECTROLYTE BALANCE IN DOGS INTOXICATED WITH NITROGEN MUSTARD

FREDERICK S. PHILIPS<sup>1</sup>, ALFRED GILMAN<sup>2</sup>, ETHOL S. KOELLE,  
BERNARD P. McNAMARA AND ROBERTA P. ALLEN

*From the Pharmacology Section, Medical Research Laboratory*

EDGEWOOD ARSENAL, MARYLAND

THE nitrogen mustards comprise a series of chemically related *bis* (2-Chloro-ethyl)amines which exhibit unique biological actions resembling in general the effects of penetrating radiations. Such properties include elicitation of chromosomal changes and genic mutation and inhibition of mitosis or selective destruction of proliferating cells and tissues (1, 2). The latter action has been found therapeutically useful in the treatment of malignant lymphomas (3-5).

Although the effects of the nitrogen mustards on certain of the actively proliferating tissues of the mammalian organism have been clearly described, the mechanisms of the lethal actions of the agents are still obscure. Thus, dogs fatally intoxicated with nitrogen mustards die within three to five days and exhibit pathological lesions in the myeloid and lymphoid elements of hematopoietic organs and in the mucosa of the gastrointestinal tract. Death is not the result of agranulocytopenia. However, the degenerative changes in the gastrointestinal tract may be directly concerned with the mechanism of lethal action. Anorexia, vomiting and diarrhea contribute to marked losses of water and electrolyte which frequently result in a reduction of extracellular and plasma fluid volumes. The associated oligemia may be the proximate cause of terminal circulatory failure (6).

Inasmuch as disturbances of water and electrolyte metabolism are manifested during the course of fatal nitrogen mustard intoxication, studies correlating changes in both intracellular and extracellular ions were undertaken in dogs. It was hoped thereby to determine whether the losses of electrolyte and fluids sustained as the result of intoxication are sufficient to account for the fatal syndrome.

## EXPERIMENTAL PROCEDURE

Twenty-three dogs were used. The experiments were divided into three series according to the agent administered and the route of administration. Animals of Series I received *tris* (beta-chloroethyl)amine percutaneously in the dose of 20 mg/kg. The free amine, freshly prepared from the hydrochloride salt, was spread as completely as possible over the shaved skin of the lumbo-sacral region. Dogs 7 and 8 were muzzled for three hours following application of mustard. At the end of this

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Received for publication October 7, 1947.

<sup>1</sup> Present address: Sloan-Kettering Institute for Cancer Research, Memorial Cancer Center, New York City.

<sup>2</sup> Present address: Department of Pharmacology, College of Physicians and Surgeons, New York City.



time their skins were decontaminated by thorough washing with soap and water. The remaining animals were muzzled for four hours and no decontamination procedure was employed.

Series IIA and IIB were originally undertaken to elucidate a possible rôle of the nervous system in the systemic effects of lethal doses of nitrogen mustards. For this purpose the highly reactive, quarternary transformation product of methyl-*bis*(2-chloroethyl)amine (1, 7), namely cationic methyl-2-chloroethyl-ethylenimonium, was employed. The effect of this agent in animals receiving intracarotid injections of 0.25 to 1.0 mg/kg. was compared with its actions in animals receiving similar doses intrajugularly. However, only dogs 12, 13 and 16 receiving 1.0 mg/kg. and 14, 15, 17 and 18 receiving 0.5 mg/kg. were used in electrolyte balance studies.

Animals used in the balance studies were fasted but allowed free access to water. All underwent a preliminary period of fasting prior to the time of administration of nitrogen mustard. In the case of dogs 1, 2, 5, and 6, this period was of 24 hours and, for the remaining animals, of 72 hours' duration.

Fluid excretions were collected under mineral oil in the presence of  $\text{H}_2\text{SO}_4$  and thymol. The cages were washed daily and the collected excreta and washings made up to a known volume. The samples were then filtered, excess thymol added and portions stored under refrigeration until completion of analysis. Since the first 4 animals studied were not catheterized, daily fluid collections in these dogs 1, 2, 5, 6 did not represent true 24-hour samples. Subsequent animals were catheterized 24 hours before the administration of mustard, immediately prior to its administration, 48 hours later, and daily thereafter.

Venous blood samples were obtained at the end of each period of fluid collection. With the exception of dogs 1, 2, 5, and 6 strict anaerobic technic was employed. A small portion of each blood sample from controls and animals of Series I was heparinized and reserved for the determination of plasma specific gravity. The remainder was allowed to clot and all other analyses were performed on serum.

#### METHODS OF ANALYSIS

Fluid excretions were analyzed for total nitrogen by the micro-kjeldahl procedure, for sodium by the method of Butler and Tuthill (8), for chloride by the Eisenmann modifications of the Volhard method as described by Peters and Van Slyke (9) and for phosphorus by the method of Fiske and Subbarow (10). Potassium was determined by modifications of the procedures of Shohl and Bennett (11), Consolazio and Talbott (12) and Harrison and Darrow (13) as follows: suitable aliquots were dried and ashed at 500°C. in vycor crucibles (30 cc. capacity). To the ash was added 0.2 cc. of 20 per cent  $\text{H}_2\text{PtCl}_6$  followed by 5.0 cc. of absolute ethanol. The precipitate which formed was stirred into a homogeneous suspension and an additional 1.0 cc. of absolute ethanol used to rinse the stirring rod. After standing for 30 minutes the alcoholic solution was separated from the precipitate by means of an inverted porcelain filter. The washing with absolute alcohol was repeated twice. The precipitate remaining in the crucible and on the face of the filter was then dissolved in a few cc. of warm distilled water and the solution was transferred through the filter by reduced pressure into a large test tube. The remainder of the procedure follows that described by Harrison and Darrow (13). Using this method 0.01 mEq. of potassium could be determined with an error of one per cent.

The serum was analyzed for sodium, potassium and chloride by the same methods described for urine as adapted to serum.  $\text{CO}_2$  was determined manometrically by the method of Van Slyke and Neill (14). Non-protein nitrogen was determined in trichloroacetic acid filtrates by the micro-kjeldahl method. Serum water was measured by difference in weight of samples after drying to constant weight at 105°C.

Plasma protein was calculated from specific gravity (15) which was determined by the falling-drop method of Barbour and Hamilton (16). Serum protein was calculated as 6.25 times the difference between total serum nitrogen and non-protein nitrogen.

#### CALCULATIONS

Changes in quantities of extracellular water and electrolyte and balance of intracellular sodium as well as the amount of potassium lost in excess of protein catabolized were calculated using the methods of Elkinton and Winkler (17) and Darrow (18) from data provided by the balance studies. For the convenience of the reader a list of the terms employed follows:

$E_{Cl}$ , volume of extracellular water assumed initially equal to 25 per cent of body weight and calculated thereafter from the balance of chloride and serum chloride concentrations;

$b_{Na}$ ,  $b_{Cl}$ ,  $b_K$ , balances of sodium, chloride and potassium, respectively;

$b'_{Na}$ , balance of intracellular sodium;

$b'_K$ , balance of potassium in excess of protein lost.

In order to emphasize the adverse effect of intoxication with nitrogen mustard additional calculations were employed to proportionate losses of sodium and chloride to initial quantities of the extracellular electrolytes present in the body. Initial quantities of extracellular sodium and chloride were estimated as the products of initial  $E_{Cl}$  and initial concentrations of sodium and chloride in extracellular water respectively. Concentrations of the ions in extracellular water were derived by correction of serum concentrations for serum water and an assumed Donnan factor of 0.96. Balances of sodium and chloride could then be expressed as percentages of the estimated initial quantities of the two electrolytes.

Calculations of potassium released in excess of protein catabolized ( $b'_K$ ) assume special importance in view of the marked deficit in this cation observed in intoxicated animals. Calculations were based on the assumption that potassium and nitrogen resulting from cell catabolism are excreted in the urine in the relative proportions present in skeletal muscle, i. e. 3 mEq/gm. N (18, 19).

Balances do not include corrections for losses in blood drawn or for changes in serum potassium since these quantities were considered insignificant by comparison with the large amounts of electrolyte excreted during the course of the experiments. Total protein catabolized was calculated from nitrogen excreted corrected for relatively minor changes in non-protein nitrogen content of body fluids.

#### CLINICAL COURSE

The control animals (1, 2, 3 and 4) were unaffected by the short period of fast. In the three series of experimental dogs, survival time of fatally intoxicated animals ranged from three to six days. In the case of the dogs of Series IIA and IIB no distinction in toxicity could be made between the two routes of administration of the ethylenimonium compound. Of the 6 animals receiving more than 0.5 mg/kg. by either the jugular or carotid route none survived. Following intrajugular administration one of 3 survived the dose of 0.5 mg/kg. and one survived 0.25 mg/kg. After intracarotid injection 2 of 4 survived 0.5 and 1 of 2 survived 0.25 mg/kg.

The clinical course of the three groups of animals was in general similar. The typical listlessness and depression which accompany nitrogen mustard intoxication were equally evident in all animals. Vomiting and diarrhea were observed in all of the three series. In Series I, all animals vomited within four to six hours after application of the amine mustard. The extent of vomiting in this group varied greatly in different animals and, inasmuch as vomitus was collected together with the urine, no accurate measure of its volume could be made. In almost every instance vomiting had ceased by the second day. On the other hand, emesis occurred within one to two

hours after injection in the dogs of Series IIA and IIB and continued intermittently until the animals died. Although no quantitative comparison was made of the extent of vomiting in Series I relative to the extent in Series IIA and IIB, there was little doubt that emesis was more severe in the two last mentioned series.

Diarrhea also complicated the experiment somewhat in that liquid feces contaminated the urine. However, diarrhea was never observed during the first 48 hours after administration of the amine mustard and was a variable feature thereafter. Of dogs in Series I, only 7, 9, 10 and 11 excreted a significant amount of watery stool. Diarrhea was evident in all the animals of Series IIA and IIB after 48 hours and was also more severe than in the animals of Series I. Watery stools undoubtedly contributed a large portion of the excretions during the latter part of the balance studies of Series IIA and IIB.

Aside from the vomiting and diarrhea there were no outstanding signs of intoxication. The animals became progressively weaker and were usually prostrate for several hours before death. Dog 8 was less affected by the dose of mustard employed presumably because it had been decontaminated with soap and water three hours after poisoning. It is possible that the animal might have survived if it had not been killed to obtain tissue for analysis before it was appreciated that its behavior was at variance with that of the other animals.

The clinical course of the surviving animals of Series IIA and IIB was not entirely uneventful. For example, dogs 15 and 18 developed diarrhea and appeared weak during the first four days after intoxication. Following this the animals recovered and ate ravenously when offered food at the termination of the experiment.

Gross pathological examinations were performed on all animals of Series I at the time of death. The findings were in keeping with those that have been reported by others. In all instances the gastrointestinal tract was examined throughout its length. Dogs 5 and 6 revealed no hemorrhagic lesions, a finding confirmed by microscopic examination.<sup>3</sup> Dogs 9, 10 and 11 showed a severe enteritis with no signs of frank hemorrhage. Although there were quantitative differences with respect to the severity of the lesions in the three dogs, the pattern of the intestinal lesions in regard to the areas most seriously affected was similar. Thus, gastritis was seen in all animals, the duodenum was little affected, enteritis was present throughout the jejunum, was almost absent in the ileum and was most marked in the colon.

#### WATER AND ELECTROLYTE BALANCE

The water and electrolyte balance of all poisoned animals was severely affected. Inspection of the results shown in table 1 reveals a striking difference in the water intake and fluid output of experimental and control animals. Whereas all animals were consistent in their water intake during the preliminary 24-hour period, the intake of the experimental animals increased markedly in response to the administration of the toxic agents. A parallel change was observed in the fluid output which was 10-fold greater in some of the experimental animals than in the controls. Reference to the performance of dogs 9, 10, 11, 12 and 13, for which daily fluid collections are

<sup>3</sup> The authors are indebted to Dr. A. M. Ginzler for the various studies of the gross and microscopic pathology of experimental animals.

TABLE 1. WATER BALANCE AND EXCRETION OF ELECTROLYTE,  
PHOSPHORUS AND NITROGEN*Dogs Poisoned at Time 0*

GROUP	DOG	PERIOD	BALANCE OF WATER		OUTPUT OF SOLUTES				
			Intake	Output	Na	K	Cl	P	N
Control	1	days	l	l.	mEq.	mEq.	mEq.	mM.	gm.
		-1 to 1	0.65	0.25	8.0	7.5	6.4	10.0	5.25
		1 to 3	0.25						
	2	-1 to 3	0.67	0.19	2.2	13.6	3.5	16.8	8.56
	3	-1 to 0	0.0	0.16	14.0	9.9	12.6	7.4	2.99
		0 to 3	0.98	1.04	22.3	37.3	33.4	31.3	10.9
	4	-1 to 0	0.27	0.14	1.3	9.8	2.6	7.9	3.41
		0 to 3	0.54	0.34	3.0	30.8	3.6	20.6	9.83
Series I	5	-1 to 2	1.66	1.53	90.2	75.7	121.2	52.6	17.9
	6	-1 to 3	3.78	2.49	58.4	118.6	97.3	75.5	32.7
	7	-1 to 0	0.19	0.06	3.4	8.0	0.6	6.1	2.41
		0 to 3	1.51	1.16	43.1	83.1	93.3	59.8	19.0
	8	-1 to 0	0.37	0.08	1.9	9.6	3.5	5.7	3.75
		0 to 3	0.93	0.49	7.3	46.3	12.7	41.5	19.0
	9	-1 to 0	0.26	0.11	2.3	6.8	6.1	9.5	3.70
		0 to 1	1.30	0.61	15.2	5.8	21.4	0.3	0.16
		1 to 2	0.91	1.10	26.1	74.9	29.6	56.5	20.3
		2 to 3	0.47	1.20	20.1	59.4	16.4	41.2	11.7
		3 to 4	0.29	0.95	2.9	44.2	29.2	19.2	6.65
	10	-1 to 0	0.25	0.12	0.7	12.8	0.4	11.0	4.42
		0 to 1	1.30	0.90	19.6	59.6	26.6	39.8	9.00
		1 to 2	1.09	0.95	26.4	56.0	31.8	37.7	12.6
		2 to 3	0.70	1.20	2.9	64.4	58.2	39.2	12.6
		3 to 3+	0.32	0.45	13.6	21.2	25.9	10.4	3.34
	11	-1 to 0	0.0	0.10	18.9	12.2	13.0	11.5	3.85
		0 to 1	0.51	0.40	23.3	4.4	32.2	0.4	0.21
		1 to 2	0.90	0.76	24.0	55.9	28.2	40.3	14.1
		2 to 3	0.59	0.92	17.2	54.8	27.6	34.2	9.78
		3 to 3+	0.0	0.80	21.9	37.2	55.5	19.5	7.63
Series IIA	12	-1 to 0	0.20	0.09	3.0	7.9	0.4	9.8	4.16
		0 to 1	1.38	2.00	94.6	51.6	168.6	24.6	5.87
		1 to 2	1.25	0.50	14.5	25.0	19.5	9.5	1.51
	13	-1 to 0	0.03	0.10	9.8	11.4	11.9	10.5	3.61
		0 to 1	0.76	0.63	30.0	27.0	45.8	22.7	4.92
		1 to 2	1.31	1.60	87.0	66.3	129.3	38.2	9.59
		2 to 3	0.62	1.04	33.5	51.2	52.2	29.7	11.2
		3 to 4	0.40	0.60	19.8	37.3	29.9	20.6	8.79

TABLE 1—Continued

GROUP	DOG	PERIOD	BALANCE OF WATER		OUTPUT OF SOLUTES				
			Intake	Output	Na	K	Cl	P	N
		days	l.	l.	mEq.	mEq.	mEq.	mM.	gm
	14	-1 to 0	0.09	0.10	trace	6.4	0.2	7.8	3.19
		0 to 2	0.60	0.94	55.9	36.8	74.2	22.8	7.12
		2 to 2+	0.0	0.28	22.5	8.7	37.3	5.4	1.65
	15	-1 to 0	0.04	0.11	19.9	14.0	11.8	11.0	4.69
		0 to 2	0.31	0.32	10.4	28.7	20.7	22.8	7.44
		2 to 4	1.56	1.67	28.5	18.7	50.9	27.5	13.2
		4 to 8	1.24	1.43	trace	79.3	14.0	69.8	31.5
Series IIB	16	-1 to 0	0.03	0.13	5.7	14.9	1.1	11.7	5.44
		0 to 1	1.44	1.12	61.3	54.0	62.2	38.2	9.32
		1 to 2	0.73	0.70	24.9	39.0	41.0	39.0	12.0
		2 to 3	0.67	0.78	22.1	49.4	29.4	40.8	16.7
	17	-1 to 0	0.17	0.09	8.3	8.1	0.4	7.2	3.73
		0 to 2	0.92	0.71	31.6	49.9	50.7	32.8	9.22
		2 to 3+	0.21	0.63	34.8	26.8	40.3	20.3	8.47
	18	-1 to 0	0.38	0.37	trace	16.2	1.5	10.3	4.6
		0 to 2	0.80	0.52	24.7	56.2	33.0	51.7	20.0
		2 to 4	0.09	0.21	trace	46.3	17.6	20.6	8.41
		4 to 8	0.79	0.34	trace	32.9	8.3	32.3	17.0

shown in table 1, indicates that a marked increase in water exchange was evident within 24 hours. Indeed, it was not unusual to note that when the muzzles were removed from experimental animals of Series I, three to four hours after poisoning, the first act of the dogs was to drink avidly. A knowledge of the renal output during this period would be of great interest. Until this is ascertained, it cannot be stated whether the polydipsia or polyuria was primary.

Inspection of tables 1 and 3 reveals that the excretion of sodium and chloride was much greater in experimental than in control dogs, indicating a marked loss of extracellular electrolyte. The animals of Series IIA and IIB sustained a greater loss than the animals of Series I. This appears to be due to the more extensive vomiting and diarrhea exhibited by the former animals. Reference to table 1 shows further that the loss of extracellular ions was apparent within 24 hours after administration of nitrogen mustard.

Although as mentioned previously vomitus contributed to all the daily collections of Series IIA and IIB only a small part of the electrolyte loss of Series I can be accounted for by emesis. In several of the dogs of Series I the volume of vomitus was negligible as compared with the total volume of the fluid collection. In almost every instance in this group vomiting had ceased by the second day whereas the excretion of sodium and chloride persisted. Lastly in dogs 9 and 11 there was complete separation in the collection of urine and vomitus due to voluntary retention of urine on the part

of the dogs during the first 24 hours of the experimental period. A comparison of the electrolyte excretion of the first day with that of the second day indicates the relative contribution to the electrolyte loss by the gastric and renal routes respectively. The extrarenal origin of the first 24-hour collection of *dogs 9* and *11* is also evident in the low values for phosphorus and nitrogen.

The extent to which diarrhea contributed to the loss of extracellular electrolyte was also greater in Series IIA and IIB than in Series I. In Series I the incidence of diarrhea was variable and in no instance were watery stools evident during the first 48 hours. Thereafter, diarrheal fluid contaminated the collections of *dogs 7, 9, 10* and *11* to a variable degree. Inspection of the amounts of sodium and potassium in the excretions after the onset of diarrhea in these animals yields the information that diarrheal fluid contributed a minor portion of the total output. *Dogs 5, 6* and *8* failed to evidence diarrhea during the course of the experiment.

Results of the calculation of changes in the volume of extracellular fluid appear in table 2. The extent to which the extracellular fluid volume was altered as the result of the administration of amine mustard reflects to some extent the balance between water intake and fluid output. Thus *dog 6* maintained extracellular fluid volume by ingestion of water. In animals which drank less the volume of extracellular fluid diminished, i.e. in the case of *dogs 5, 9* and *13*. Other animals exhibited an intermediate course. Obviously in those cases in which extracellular volume was largely maintained in the face of the loss of extracellular electrolyte, the concentration of extracellular electrolyte was markedly decreased.

In table 3 it may be seen that the intoxicated animals with certain exceptions lost 10 or more per cent of initial extracellular sodium and even larger portions of initial extracellular chloride through the kidneys and gastrointestinal tract. This is in marked distinction to the control animals and to *dogs 15* and *18* which survived as well as *dog 8* which for reasons explained above may have absorbed a smaller amount of amine mustard. A study of table 3 reveals further that the extent of excretion of sodium and chloride was greater in animals of Series IIA and IIB than in those of Series I. It is evident, therefore, that the systemic actions of methyl-beta-chloroethyl-ethylenimmonium caused a more severe loss of extracellular electrolyte than resulted from fatal intoxication with *tris*(beta-chloroethyl)amine. This appears to be associated with the extensive vomiting and diarrhea which was exhibited by the animals receiving the chlor-imine. It is also important to observe that fatal intoxication with LD<sub>50</sub> doses of the chlor-imine in *dogs 14* and *17* resulted in losses of sodium and chloride similar in degree to those caused either by higher dosages of the same agent or by lethal amounts of *tris*(beta-chloroethyl)amine. On the other hand, *dogs 15* and *18*, which survived after receiving LD<sub>50</sub> doses of the chlor-imine, showed only moderate though definite increases in electrolyte excretion.

*Balance of Intracellular Sodium.* The excretion of chloride exceeded that of sodium by significant amounts in all poisoned dogs with the possible exception of *8* (table 3). During the first two days of intoxication the small excess could be attributed to the amount of vomitus in the fluid excretions. During the terminal portion of the observation periods, however, it was not uncommon for chloride to be excreted far in excess of sodium. This is evident in table 1 where, in the cases of *dogs*

TABLE 2. WEIGHT AND ANALYSES OF SERUM AND BLOOD IN FASTING DOGS INTOXICATED WITH NITROGEN MUSTARD AND IN THEIR CONTROLS. CALCULATION OF EXTRACELLULAR FLUID VOLUME

*Dogs Poisoned at Time 0*

GROUP	DOG	TIME	WEIGHT	CONCENTRATION IN SERUM						PLASMA OR SERUM PROTEIN	ECI
				Na	K	Cl	Total CO <sub>2</sub>	H <sub>2</sub> O	NPN		
		<i>day</i>	<i>kg.</i>	<i>mEq/l.</i>	<i>mEq/l.</i>	<i>mEq/l.</i>	<i>mM/l.</i>	<i>gm/100 gm.</i>	<i>mg/100 cc.</i>	<i>gm/100 cc.</i>	
Control	1	-1	13.2	154	6.0	114					
		3	12.5	154	5.5	115					
	2	-1	9.8	155	5.6	119				6.3	2.45
		3	9.0	149	6.3	116				5.4	2.49
	3	0	23.56	141		106	25.1	92.3	17.0	6.0	5.88
		3	22.82	135		105	25.4	93.2	24.2	5.7	5.76
	4	0	11.21	136		105	23.3	91.6	25.0	7.2	2.80
		3	10.94	136		100	22.4	92.4	28.8	6.9	2.91
Series I	5	-1	16.1	141	5.1	108				6.4	4.02
		2	14.2	145	5.4	94.5				8.3	3.44
	6	-1	13.6	144	4.8	108				6.4	3.40
		3	12.5	127	5.8	82.5				6.7	3.41
	7	0	11.42	140		106	22.6	92.3	28.2	6.1	2.86
		3	10.06	134		84.5	33.9	91.8	49.6	5.4	2.61
	8	0	14.17	141		106	24.0	92.5	49.7	6.1	3.54
		3	13.85	137		101	24.6	92.7	31.4	6.1	3.59
	9	0	17.21	147	4.0	114	21.7	91.6	21.7	7.3	4.30
		2	16.33	137		102	20.5	91.3		7.2	4.35
		3	15.37	142	5.1	103	23.2	90.7	52.2	8.3	4.13
		4	14.31	149	4.4	111	21.7	90.2	107	8.7	3.58
	10	0	14.29	143	4.6	111	20.6	92.3	22.5	6.9	3.57
		2	13.61	132	4.1	98.9	20.8	92.2	32.3	6.7	3.47
		3	12.87	131	4.1	92.6	23.5	91.8	48.3	7.3	3.15
	11	0	17.72	145	4.5	108	22.3	91.3	22.6	7.1	4.43
		2	16.95	139	3.8	100	24.1	91.3		7.5	4.26
		3	16.19	140	4.4	96.3	24.7	91.3	37.9	8.1	4.15
Series IIA	12	0	12.10	146		108	23.1	92.8	32.0	5.5	3.13
		2	11.00	134		61.6	33.7	91.0	177	6.9	2.70
	13	0	13.19	146		111	20.4	91.8	29.5	6.3	3.30
		2	12.22	135		82.8	33.3	90.9	36.2	7.2	2.54

TABLE 2—Continued

GROUP	DOG	TIME	WEIGHT	CONCENTRATION IN SERUM						PLASMA OR SERUM PROTEIN	ECI
				Na	K	Cl	Total CO <sub>2</sub>	H <sub>2</sub> O	NPN		
		day	kg.	mEq/l.	mEq/l.	mEq/l.	mM/l.	gm/100 gm.	mg/100 cc.	gm/100 cc.	l.
		3	11.68	129		67.9	41.2	91.1	36.0	7.4	2.44
		4	10.98	115		66.1	39.4	91.6	21.1	7.1	2.12
	14	0	11.1	144		104	24.3	92.7	33.5	5.7	2.78
		2	10.0	138		92.1	30.0	91.6	40.5	6.7	2.40
	15	0	12.7	150		110	25.2	92.3	23.5	6.2	3.18
		2	11.9	139		104	25.7	91.9	27.6	6.6	3.16
		4	11.2	135		88.1	33.2	91.9	44.6	6.8	3.22
		8	10.3	137		87.5	31.8	92.7	39.5	6.1	3.13
Series IIB	16	0	14.68	152		111	23.1	92.3	32.1	5.9	3.67
		2	13.38	135		90.2	24.7	91.8	71.7	6.8	3.49
		3	13.27	129		83.2	27.0	92.2	69.5	6.3	3.49
	17	0	8.6	141		106	23.6	93.3	30.8	5.2	2.15
		2	8.2	135		82.1	31.3	91.9	114	5.9	2.19
	18	0	13.3	144		113	20.7	93.2	37.7	5.3	3.32
		2	13.0	141		106	22.4	92.9	34.0	5.5	3.24
		4	12.4	140		106	22.7	93.0	30.8	5.5	3.08
		8	11.3	139		107	23.6	93.0	32.4	5.6	2.99

9 and 10, the extreme condition was observed in which the excretions were almost sodium-free and yet contained large amounts of chloride. Nevertheless, the fact that chloride was excreted in excess was not uniformly reflected in all dogs by a significant change in total serum CO<sub>2</sub> (see dog 9, table 2). This may indicate a passage of sodium into cells in exchange for potassium. Indeed, calculation reveals gains of intracellular sodium in many of the experimental animals following intoxication as well as a direct relationship between increases of total serum CO<sub>2</sub> and of the calculated value,  $(b_{Na}-b_{Cl}) - b'_{Na}$  (table 3). The latter value may be taken to represent the calculated maximal increase in the available base of extracellular fluid expected to result from the excretion of chloride in excess of sodium. The only notable exception to the apparent correlation are the results from dog 13 between zero and four days after intoxication. Even in this animal the correlation fails only after the third day of intoxication. Inspection of the values for  $b'_K$  in table 3 also reveals that most of the experimental dogs lost sufficient potassium from cells, beyond those amounts resulting from catabolism, to account for the instances in which significant gains in intracellular sodium were calculated.

*Potassium, Phosphorus and Nitrogen Excretion.* Intoxicated animals excreted potassium, phosphorus and nitrogen greatly in excess of controls (table 1). In sev-



eral instances such as *dogs 10, 12, 13* and *16* daily collections of excreta revealed that the enhanced output of potassium, phosphorus and nitrogen was evident within 24 hours after intoxication. Voluntary retention of urine on the part of *dogs 9* and *11* undoubtedly accounts for the low values obtained analytically in the collections of the first 24 hours.

The increased excretion of intracellular cation in experimental animals can in part be attributed to an increased cellular catabolism. This might be expected to

TABLE 3. COMPARISON OF THE BALANCE OF INTRACELLULAR SODIUM WITH THE EXCRETION OF CHLORIDE IN EXCESS OF SODIUM, WITH CHANGES IN SERUM CO<sub>2</sub>, AND WITH EXCRETION OF POTASSIUM IN EXCESS OF PROTEIN CATABOLIZED. TOTAL ELECTROLYTE LOSSES IN EXPERIMENTAL AND CONTROL DOGS

SERIES	DOG	PERIOD	$b_{Na}-b_{Cl}$	$b'_{Na}$	$(b_{Na}-b_{Cl})-b'_{Na}$	CHANGE IN TOTAL SERUM CO <sub>2</sub>	$b'$		PORTION OF INITIAL EXTRACELLULAR ELECTROLYTE LOST	
									Na	Cl
		days	mEq.	mEq.	mEq.	mM/l.	mEq.	mEq/kg/day	%	%
I	5	-1 to 2	+31	-17	+48		-22	-0.48	15	25
	6	-1 to 3	+39	-1	+40		-21	-0.40	11	23
	7	0 to 3	+50	+8	+42	+11.3	-22	-0.68	10	27
	8	0 to 3	+5	0	+5	+0.6	+5	+0.12	1.4	3.1
	9	0 to 4	+32	+32	0	0.0	-42	-0.65	10	17
	10	0 to 3	+68	+51	+17	+2.9	-70	-1.72	12 <sup>1</sup>	32 <sup>1</sup>
	11	0 to 3	+24	+4	+20	+2.4	-30	-0.59	13 <sup>1</sup>	26 <sup>1</sup>
II A and B	16	0 to 3	+24	+5	+19	+3.9	-19	-0.46	19	29
	12	0 to 3	+79	-18	+97	+10.6	-22	-0.95	23	50
	13	0 to 3	+77	+22	+55	+20.8	-67			
		0 to 4	+87	+80	+9	+19.0	-82	-1.69	34	62
	14	0 to 2	+18	+10	+8	+5.7	-15	-0.71	19 <sup>2</sup>	34 <sup>2</sup>
	15	0 to 4	+33	+4	+29	+8.0	+15	+0.31	7.9	18
	17	0 to 2	+19	-29	+48	+7.7	-8	-0.48	21 <sup>1</sup>	31 <sup>1</sup>
	18	0 to 4	+26	+24	-2	+2.0	-20	-0.39	5.1	12
Control	2	-1 to 3						+0.32	0.5	1.2
	3	0 to 3						-0.02	2.5	4.7
	4	0 to 3						-0.02	0.8	1.2

<sup>1</sup> Calculated for 0 to 3 + days.    <sup>2</sup> Calculated for 0 to 2 + days.

be the result of the known cytotoxic actions of amine mustards and is evidenced by the enhanced excretion of nitrogen and phosphorus in experimental dogs. However, an analysis of the amount of potassium in the fluid excretions in relation to the amount of nitrogen reveals that the experimental animals excreted intracellular cation in excess of those quantities which could be accounted for solely on the basis of enhanced cellular catabolism.

Values for potassium lost as the result of processes other than catabolism are shown in table 3. The  $b'_K$  values shown in table 3 for experimental animals may be compared with the values derived from 17 periods of collection from animals prior to intoxication and from controls which ranged between -0.18 and  $\pm 0.36$  mEq/kg/day

and averaged  $\pm 0.08$ . It is evident that in all experimental animals with the exception of *dogs 8* and *15* the losses of potassium during periods following intoxication were excessive.

In view of the losses of excess potassium suffered by most of the experimental dogs it is worthwhile to consider briefly the exceptional dogs, *8* and *15*. For reasons outlined previously the former animal probably received a sub-lethal dose of nitrogen mustard; the latter animal survived the period of intoxication. Both animals sustained no significant losses of potassium in excess of that presumed to result from cellular catabolism. It is, therefore, of interest to note that *dog 8* likewise showed no significant increase over the control period in water intake, fluid output and sodium and chloride excretion. Moreover, *dog 15* exhibited changes in these excretory processes to a lesser degree than did experimental animals which failed to survive. The moderate increase in excretion of potassium and nitrogen found in *dogs 8* and *15* can be attributed to a moderate dissolution of lymphoid and myeloid tissue. It may finally be noted that *dog 18*, a surviving animal like *15*, exhibited losses of excess potassium which were intermediate between those of controls and those of non-survivors.

*Changes in Plasma Protein.* It will be noted in the data of table 2 that a reciprocal relationship exists between extracellular fluid volume and plasma protein concentration (the only notable exception is *dog 7*). This is particularly evident in the response of the plasma protein concentration of *dog 9* in that during the first 48 hours when extracellular fluid gained 0.08 liters plasma protein fell slightly. During the next 48 hours the decline in extracellular fluid volume was associated with a marked increase in plasma protein concentration. The reciprocal relationship between plasma protein concentration and the volume of the extracellular fluid suggests that the impermeability of the capillary wall to protein remained unimpaired.

#### DISCUSSION

The above data present evidence for *a*) loss of extracellular electrolyte, *b*) loss of intracellular electrolyte and *c*) increased catabolism as the result of fatal intoxication with nitrogen mustard. However, the rôle of electrolyte and water imbalances in the sequence of events involved in the fatal syndrome is not clear. To be sure, there is ample evidence justifying the conclusion that death is associated with a marked inability to retain extracellular electrolyte. Regardless of the mechanism, whether through emesis, diarrhea or failure of renal reabsorption, the loss during the first three to four days following intoxication of 10 or more per cent of total extracellular sodium and 17 or more per cent of total extracellular chloride is associated with a fatal outcome. Moreover, animals which survive LD<sub>50</sub> doses show a transient loss of sodium and chloride which appears significantly increased over that of starving controls but which fails to assume the proportions of the electrolyte disturbances associated with death. Nevertheless, the extent of the loss of extracellular electrolyte by itself seems insufficient to explain the fatal outcome of intoxication in all animals. In experimental animals losses of one third to one half of total extracellular electrolyte are usually considered fatal (20). Of the present animals none of Series I and only a few individuals of Series IIA and IIB sustained losses to this extent. Moreover, it is difficult to attribute death solely to a loss of extracellular electrolyte when in an

extensive series of experiments Smith and co-workers have shown that supportive therapy designed to maintain extracellular electrolyte and fluid intact, benefits but fails to save a significant number of animals from the systemic effects of nitrogen mustards (6).

It is of interest to consider the mechanism by which extracellular electrolyte is lost as the result of nitrogen mustard poisoning. A portion was lost as the result of vomiting; another fraction was lost later in the course of poisoning from the intestinal tract due to diarrhea. However, in the animals of Series I which received *tris*(beta-chloroethyl)amine the greatest amount appeared to have been excreted by the kidneys. This must be attributed to a renal insufficiency in the reabsorption of sodium and chloride. The wastage of extracellular electrolyte exhibited by the poisoned dogs of Series I is not unlike that encountered in adrenal insufficiency. However, there was no evidence of any rise in serum potassium despite the fact that large amounts of this cation were made available for excretion during the experimental period. Moreover, no pathological change in the adrenal has been observed to support such a thesis. One is left with the conclusion that *tris*(beta-chloroethyl)amine produces a functional disturbance of the kidney of such magnitude as to interfere with homeostasis. Presumably the animals of Series II which received methyl-beta-chloroethylethylenimonium were similarly affected although for reasons advanced previously renal losses could not be distinguished readily in this group from losses due to vomiting and diarrhea.

Alteration of the volume of the intracellular fluid likewise seems insufficient to account for the morbid changes which occur during fatal intoxication. Approximate values for changes of cell fluid volume may be estimated as the difference between the observed changes in weight and the calculated changes in the volume of extracellular fluid during the period of intoxication. Such calculations reveal depletions of cell fluid in all of the animals which succumbed with the exception of *dog 9* of not more than 10 per cent of initial body weight. *Dog 9* lost cell fluid to the extent of about 13 per cent of initial body weight, a result which is probably associated with the fact that this animal exhibited the most negative water balance. These values appear to be well within the limits compatible with viability in dogs which have been subjected to chronic deprivation of food and water (21). Nevertheless, the values for cell fluid loss in the present animals were significantly higher in poisoned dogs than in their starving controls.

Lastly the contribution of the loss of intracellular potassium to the lethal action of the mustards should be considered. As already stated this can only partly be accounted for on the basis of increased catabolism. It is important to examine the possibility that the loss of potassium in excess of protein catabolized can be associated with the cellular dehydration noted in poisoned animals. Elkinton and co-workers (17, 21, 22, 23) have shown that significant loss of excess potassium is obtained in dogs subjected to procedures which lead to depletion of cell water. The depletion may be instigated diversely by chronic deprivation of food and water, by parenteral administration of hypertonic solutions of NaCl, by removal of fluid from the peritoneal cavity after intraperitoneal administration of hypertonic solutions of NaCl or glucose, or by urea diuresis. All of the above procedures cause a primary loss of extracellular fluid or an increase in concentration of extracellular electrolyte. However, in the

present animals loss of cell water occurred usually in the face of a loss of extracellular electrolyte and a reduction in the osmotic pressure of extracellular fluid. In view of these facts it is not surprising that inspection of tables 1, 2 and 3 reveals no correlation between the balance of excess potassium and either the balance of water or the extent of change of intracellular fluid as estimated from changes in weight and  $E_{Cl}$ . Indeed, *dog 6* showing the most positive water balance and a moderate change of body weight and fluid volume exhibited a negative balance of excess potassium (calculated in mEq/kg/day) which differed little from that of *dog 9* showing the most negative water balance and the greatest loss of cell fluid.

Another possible explanation for the loss of intracellular cation is suggested by the recent clinical and experimental observations that drastic changes in the composition of the extracellular environment either induced experimentally or resulting from excessive therapy with sodium chloride or from severe diarrhea may often be accompanied by unexplained losses in intracellular potassium (20, 24). The deficits of intracellular potassium are often associated with abnormally high concentrations of intracellular sodium and serum bicarbonate. However, in the present experimental animals which consistently underwent severe changes in the composition of the extracellular fluid due to emesis, diarrhea and renal impairment of electrolyte reabsorption, the loss of potassium cannot be correlated with the extent of the loss of extracellular electrolyte or change in the bicarbonate concentration of serum (Series I versus Series II, table 3). Furthermore, in the above experiments excretion of potassium in excess of nitrogen occurred as early as the first experimental day at a time when distortion of the extracellular fluid had not yet become marked.

It is tempting to attribute the loss of intracellular potassium to a direct cytotoxic action of nitrogen mustard which affects metabolic activity so that the cell is unable to maintain the integrity of its cationic structure. This type of action would presumably result in the exchange of sodium and potassium across the cell membrane. Potassium in an extracellular site would be preferentially excreted by the renal tubule with an equivalent of anion. The close correlation between  $b'_K$  and  $b_{Na} - b_{Cl}$  (table 3) is in keeping with such a sequence of events. Although the correlation between  $b'_K$  and  $b'_{Na}$  is less obvious, nevertheless, significant increases in intracellular sodium are observed in the dogs which show the greatest loss of excess potassium.

That an intracellular potassium loss would contribute further to the cytotoxic action of nitrogen mustard appears likely. The observations of Darrow on the importance of replacing potassium loss sustained in diarrhea are pertinent in this regard (20). Thus a cytotoxic agent could presumably initiate a vicious cycle of events in which the biochemical lesion initially produced by a toxic agent could be sustained by progressive depletion of potassium until ultimate functional failure of the cell. In the case of nitrogen mustard poisoning there may be the further contribution of distortion in the chemistry of the extracellular fluids secondary to impaired renal function, diarrhea and vomiting. Although no single mechanism would be sufficient to cause death, the combination presumably results in conditions incompatible with survival.

#### SUMMARY

Systemic intoxication by *tris*(beta-chloroethyl)amine and methyl-beta-chloroethyl-ethylenimonium in dogs is characterized by an extensive loss of extracellular and

intracellular electrolyte and a greatly increased fluid intake and output. The loss of extracellular electrolyte may be due in part to vomiting and diarrhea but also results from a renal defect in the reabsorption of sodium and chloride. A large portion of the intracellular electrolyte lost can be accounted for on the basis of increased catabolism, presumably for the most part of lymphoid and myeloid tissues. The remainder of the potassium excreted is in excess of protein catabolized. The extensive loss of electrolyte exhibited by animals which receive lethal doses appears to be an essential component of the fatal syndrome. However, it is not possible to conclude that the deficits of both extracellular and intracellular electrolyte are the direct cause of death.

The loss of potassium in excess of the amounts appearing as the result of enhanced catabolism may be associated with a unique cytotoxic action of nitrogen mustards. However, other possible causes for the unexplained excesses in potassium excretion are discussed.

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## CORRIGENDA

Volume 153, page 508. Line 5: The designation  $C_0^+$  should read  $C_0^*$ .

Line 10: The equation should read  $C_t^* = C_0^* e^{-Rt}$ .

Volume 154, page 428. In title and throughout text, "Herring-Breur" should read "Hering-Breuer".



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# THE AMERICAN JOURNAL OF PHYSIOLOGY

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*Published by*  
THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOLUME 155

December 1, 1948

NUMBER 3

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## WATER INGESTION AND EXCRETION IN RATS UNDER SOME CHEMICAL INFLUENCES<sup>1</sup>

E. F. ADOLPH

*From the Department of Physiology, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

TWO different concepts prevail concerning the regulation of water turnover in mammals. One is that intake is a haphazard process and that accurate corrections of water content are performed by excretion. The other is that ingestion, while intermittent, is as sensitive to modifications of body water content as is excretion. The following experiments were designed to test some modifications that could be elicited in water intake and in water output. The ulterior object of the investigation was to elucidate some of the factors in thirst, the urge to drink. Factors in water intake and output were to be studied separately and together.

Experiments, conducted upon male rats whose mean body weight was 220 gm., were of three sorts. In Series I, rats were furnished diluted milk (2.6% solids) so that large volumes were ingested by them (1). The amounts of ingesta (from tube-tipped graduated cylinders) and of collectable urine were measured in hourly periods after intramuscular injection of a chemical agent. In this manner certain agents that inhibit water turnover were identified. In order to find (Series II) whether ingestion alone was influenced by the agents, rats kept on a diet of dried whole milk were without drinking water for 48 hours and were injected with agents shortly before drinking of distilled water was again allowed. In order to ascertain (Series III) whether water excretion itself was inhibited, rats kept on dried milk up to the hour of experiment were given by stomach tube six doses of 5 per cent of the body weight of water, at 30-minute intervals. Urine was collected but no drinking water was available during this test.

*Series I.* Rats that were furnished 2.6 per cent milk solids in distilled water as the only source of intake ingested approximately their own body weights of liquid every day. The mean fluid intake and urinary output were quite uniform hour

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Received for publication October 10, 1948.

<sup>1</sup> The investigation was aided by a contract between the Aeromedical Laboratory, U. S. Air Forces, and the University of Rochester. Technical assistance was rendered by S. Parmington, J. Northrop and L. Heilbrunn.



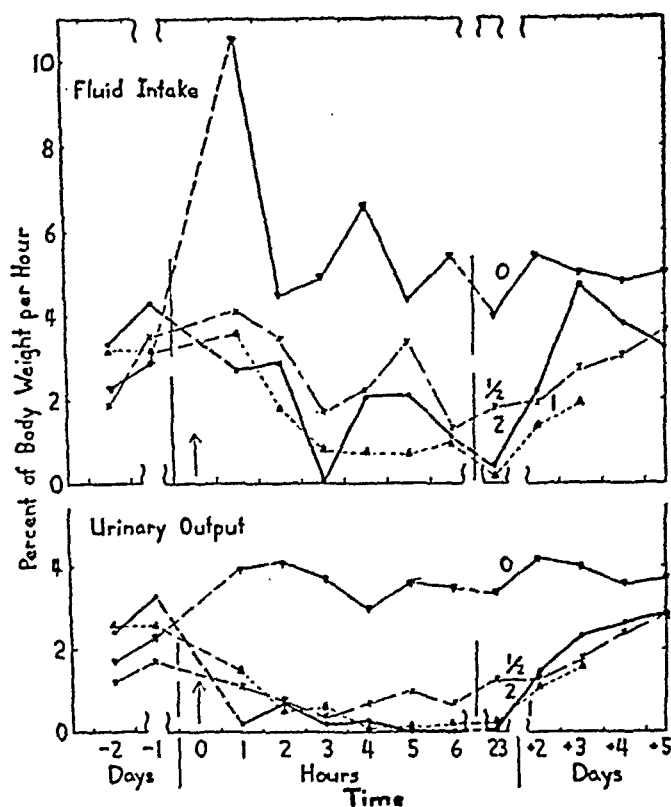
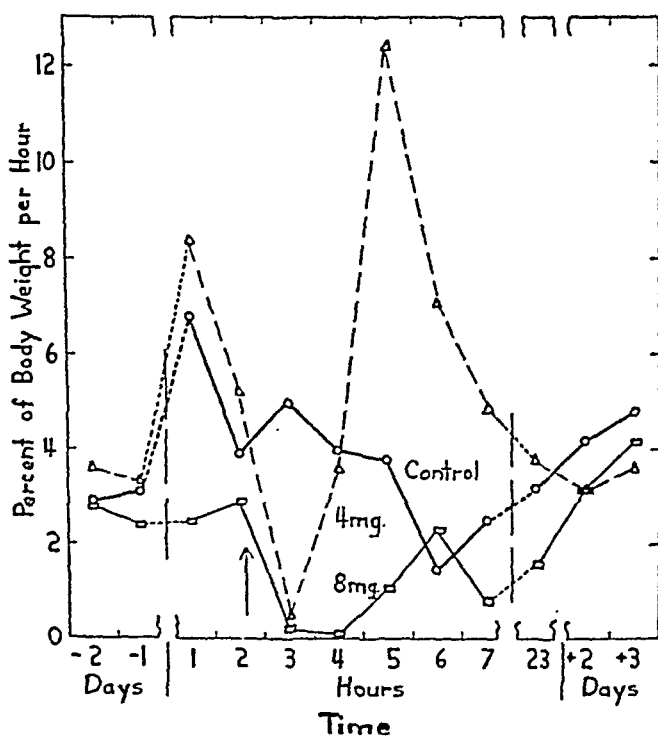


Fig. 1. INTAKE OF DILUTED MILK AND OUTPUT OF URINE by rats injected with pitressin in oil at zero time. Each line represents the mean observed in 7 tests. The first 2 and last 4 points of each line represent daily averages; the 23-hr. point is the mean of 17 hr.; the intermediate points are for single hr. Pitressin tannate in oil was injected intramuscularly at the arrow in doses of 0,  $\frac{1}{2}$ , 1 or 2 U/rat. The excessive intake in the first hr. followed the unavailability of milk mixture for about one hr. before injection. All the doses ( $\frac{1}{2}$ , 1 or 2 U.) evidently inhibited both intake and output for more than 24 hr.

Fig. 2. INTAKE OF DILUTED MILK by rats injected with pilocarpine at 2 hr. Each line represents the mean result of 4 tests. The first 2 and last 2 points represent daily av.; the 23-hr. point is the mean of 16 hr.; all other points are single hr. Pilocarpine greatly suppressed the fluid intake at first, but the 4 mg. injection greatly enhanced the intake in several later hours.



after hour (fig. 1). Both were temporarily suppressed by administration of sufficient atropine, pilocarpine (fig. 2) and postpituitary extracts (fig. 1). Other substances tested, namely acetylcholine and epinephrine, had no significant effect (table 1).

It is widely recognized that pilocarpine and atropine antagonize one another in many of their physiological effects. When the two were administered successively to the same rats (table 1), water turnover was in most instances reduced. Since

TABLE 1. INFLUENCES OF SEVERAL AGENTS UPON WATER EXCHANGES  
(Means and Standard Errors)

Series I

AGENT	DOSE/RAT (INTRAMUSCULARLY)	NO. OF TESTS	OUTPUT OF URINE	INTAKE OF 2.6% MILK	INTAKE IN SECOND HOUR	INTAKE
			% wt/hr. in first 24 hrs.	% wt/hr. in first 24 hrs.	% wt.	% wt/hr. in first 5 hrs.
Control		15	3.1 $\pm$ 0.43	4.2 $\pm$ 0.48	5.5 $\pm$ 1.11	5.2 $\pm$ 0.38
Acetyl- choline	300 $\mu$ g. 600 $\mu$ g.	4	2.2	3.0	2.2	2.3
Doryl	50 $\mu$ g. 200 $\mu$ g. 600 $\mu$ g.					
Atropine	17 $\mu$ g. 50 $\mu$ g.	4 4	2.3 2.2 $\pm$ 0.4	3.2 2.9 $\pm$ 0.4	2.2 $\pm$ 0.4	2.4 2.0 $\pm$ 0.5
Atropine, then Pilo- carpine	17 $\mu$ g. + 0.5 mg. 50 $\mu$ g. + 4.0 mg. 100 $\mu$ g. + 4.0 mg.	2 2	3.4 2.2	4.5 2.9	3.4 2.2	1.0
Epinephrine in oil	300 $\mu$ g.	3	2.9	3.9	2.9	3.4
Pilocarpine	0.5 mg. 1.0 mg. 2.0 mg. 4.0 mg. 8.0 mg.	2 4 4	3.0 3.2 0.96	3.9 4.8 (fig. 2) 1.5 (fig. 2)	3.0 3.2 0.96	3.7 5.7 0.9
Pitressin in oil	0.5 U. 1.0 U. 2.0 U.	7 7 7	1.2 $\pm$ 0.06 0.26 $\pm$ 0.32 0.08 $\pm$ 0.14	2.1 $\pm$ 0.17 0.53 $\pm$ 0.08 0.56 $\pm$ 0.06	1.2 $\pm$ 0.06 0.26 $\pm$ 0.32 0.08 $\pm$ 0.14	3.0 $\pm$ 0.86 1.5 $\pm$ 0.15 1.3 $\pm$ 0.26
Pituitrin aqueous	1 to 3	22	2.5 $\pm$ 0.68	3.0 $\pm$ 0.77	2.5 $\pm$ 0.68	3.4 $\pm$ 0.68

each of the two agents alone suppressed the turnover anyway, it would be unlikely that when combined they would antagonize one another in this phenomenon.

Dilution of food, therefore, inducing a continuous voluntary turnover of large quantities of water, furnished a basic pattern of rapid water exchange. Three chemical preparations in appropriate doses were capable of suppressing part of the excessive turnover. The question then remained as to whether the agents acted upon some

identifiable part of the water exchange; it was partially answered in Series II and III below.

*Series II.* Rats that had been without water (but with dry food) for 48 hours lost about 9 per cent of the body weight. When then allowed to drink they ordinarily drank enough water in one hour to make up about three fourths of the weight that had been lost (fig. 3), but when certain doses of pilocarpine were given, intake was severely inhibited. The inhibition did not last through a whole day, however, and recovery from water deficit was largely achieved before food was again furnished to the rats.

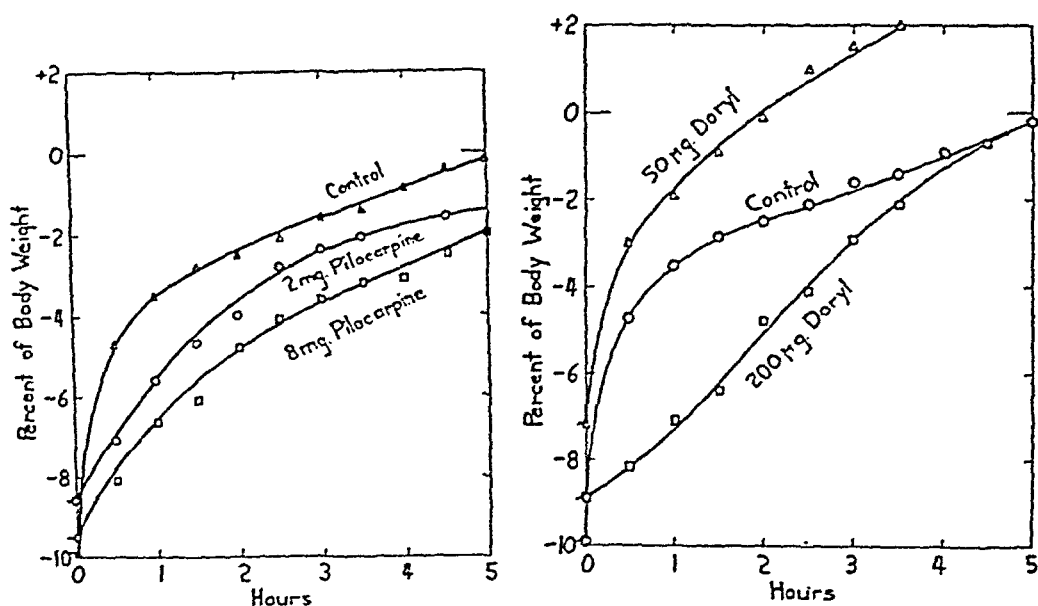


Fig. 3 (left). CUMULATIVE INTAKES of distilled water by rats injected with pilocarpine 0.5 hr. before water was first allowed at zero time. Rats had been deprived of water but allowed dry food (whole milk powder) for 48 hr. previously. Body wt. 48 hr. ago was taken as zero. Each point is the mean of 29 (0 dose), 9 (2 mg.) and 7 (8 mg.) tests. Drinking was inhibited in the first half hour by both doses.

Fig. 4 (right). CUMULATIVE INTAKES of distilled water by rats that had been deprived of water during the previous 48 hr. They were allowed food during that period, but not after zero time. The body wt. 48 hr. previously was taken as zero wt. Controls, 29 tests; 50 µg. doryl, 4 tests; 200 µg. doryl, 6 tests, animals prostrated for 2 hr., which accounts for their small initial intake. Doryl was injected into a leg muscle 0.5 hr. before zero time at which water was first allowed.

Of the hormone preparations tested, only pilocarpine and doryl (carbaminoylecholine) had significant influences upon water ingestion (table 2). The manners of action of the two agents were markedly different. After pilocarpine the animals mouthed the drinking tubes but took very little from them. Often they withdrew to other parts of the cages, but soon approached the tubes again. This may be designated as a behavior of frustration. After doryl (fig. 4) the animals were prostrated, to the point where they were physically unable to drink, by a dose sufficient to inhibit drinking at all. Pilocarpine could be said to exert an effect upon drinking, but doryl an effect upon all neuromuscular activities including drinking. In this way it is demonstrated that of the three agents that reduced the rate of water turnover,

one of them (pilocarpine) influences water intake under conditions where intake is not inhibited by the accumulation of a water excess in the body. In dehydrated rabbits a similar inhibition of drinking was reported by Pack (2). In dehydrated men a lesser dose of pilocarpine had no influence upon drinking (3). The effects of

TABLE 2. INFLUENCES OF SEVERAL AGENTS UPON WATER EXCHANGES  
(Means and Standard Errors)

AGENT	DOSE/RAT (INTRAMUSCULARLY)	SERIES II		SERIES III	
		NO. OF TESTS	INTAKE OF WATER AFTER 48 HR. WITHOUT WATER	NO. OF TESTS	OUTPUT OF URINE AFTER 6 x 5% WT. IN 3 HRS.
			% wt. in first hr.		% wt. in 4th hr.
Control		29	6.4 $\pm$ 0.38 (fig. 3)	25	6.0 $\pm$ 0.25
Acetylcholine	300 $\mu$ g. 600 $\mu$ g.	4	6.1	4	6.4 $\pm$ 0.6
Doryl	50 $\mu$ g. 200 $\mu$ g. 600 $\mu$ g.	4 6 4	5.3 (fig. 4) 1.8 Prostrated Killed	5	4.9 $\pm$ 0.4
Atropine	17 $\mu$ g. 50 $\mu$ g.	4	5.0	3 6	6.3 $\pm$ 1.3 5.0 $\pm$ 0.4 (fig. 3)
Atropine, then Pilocarpine	17 $\mu$ g. + 0.5 mg. 50 $\mu$ g. + 4.0 mg. 100 $\mu$ g. + 4.0 mg.	4	5.3	2	4.1 $\pm$ 2.1
Epinephrine in oil	300 $\mu$ g.				
Pilocarpine	0.5 mg. 1.0 mg. 2.0 mg. 4.0 mg. 8.0 mg.	3 2 9 3 7	7.4 7.1 3.0 3.9 $\pm$ 1.0 (fig. 3) 2.9 $\pm$ 0.8 (fig. 3)	3  4 4	5.6 $\pm$ 0.4  5.5 $\pm$ 0.8 5.0 $\pm$ 0.7
Pitressin in oil	0.5 U. 1.0 U. 2.0 U.	5	5.5		
Pituitrin aqueous	1 to 3 U.	4	4.5		

pilocarpine in all species are transient and show themselves only when observations are made in periods of an hour or less.

Other effects of pilocarpine and doryl were visible. Both of them in all the doses tested (but none of the other agents) produced the well-known secretion of red pigment (protoporphyrin) in eyes and nose. Pilocarpine in doses of 4 and 8 mg. (and

no other agents) produced diarrhea; this was not seen until one hour after drinking began. Both substances (and no other agent) produced drooling salivation. It has been supposed by other investigators that the presence of copious saliva in the pharynx is important in the inhibition of water drinking (e.g. Cannon, 4). We observed as much salivation after 50  $\mu$ g. of doryl and after 0.5 mg. of pilocarpine, which did not inhibit drinking, as after larger doses of each that did suppress drinking. This fact seems to constitute clear evidence that water intake is not diminished merely by having the throat flooded with saliva.

Postpituitary agents have sometimes been supposed to modify the urge to drink. Physiologists familiar with the syndrome of diabetes insipidus suggest that these agents will generally reduce the amounts drunk. Those who think of pituitrin as

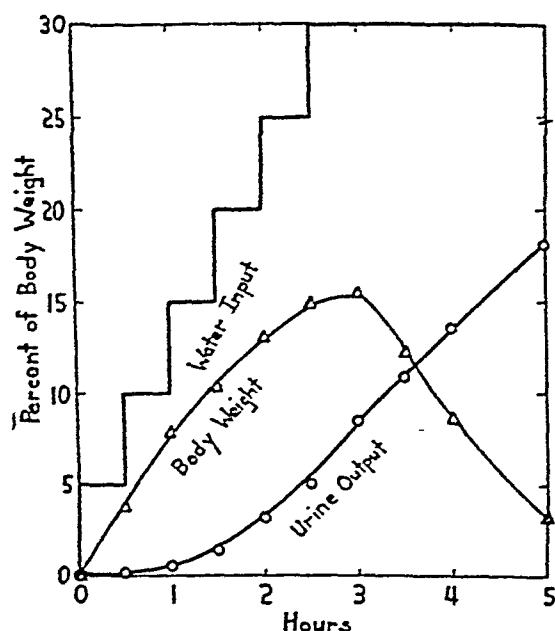


Fig. 5. CUMULATIVE EXCHANGES OF WATER and body wt. in rats to which water was forced by stomach tube in 6 portions. Each point is the av. of 6 tests; 50  $\mu$ g. of atropine were injected 0.5 hr. before zero time. The substance injected produced no difference from controls.

antidiuretic are likely to suggest that drinking will be enhanced by it. The present experiments show that neither effect is significantly realized in normal rats.

*Series III.* The capacity to excrete water at high rates was tested by administering large volumes of water by stomach tube. Thirty per cent of the body weight of water was given within three hours. After the last dose the body weight was usually 13 per cent in excess and a rate of urine formation of 6 per cent of the body wt./hr. was regularly reached. When atropine was given, no significant modification occurred in the rate of urinary excretion even for an hour or two (fig. 5). Not all of the agents under consideration were tested here (table 2) because numerous tests of antidiuresis have been reported by other investigators.

The antidiuretic properties of postpituitary extracts are well known (5, 6), but it may be recalled that in some doses the extracts enhance urinary flow (7). Epinephrine has not been found to be antidiuretic in rats; in some doses it even accelerates diuresis (8). Acetylcholine is believed to oppose diuresis by exciting the postpituitary gland to secrete antidiuretic substance (9, 10); though after injection its influence is short-lived, perhaps due to its rapid inactivation.

The upshot of the investigation is that atropine, pilocarpine and postpituitary substances may temporarily reduce the turnover of excessive water by rats. Pilocarpine in large doses inhibits drinking, pituitrin inhibits excretion and atropine succeeds in affecting water turnover only in the second hour after it is injected. The results serve as a warning against too literal a partitioning of the diverse water exchanges.

#### COMMENT

The tests here reported demonstrate clearly that rapid turnovers of water may be limited at intake as well as at output. Whenever intake of dilute food mixture is reduced (by pilocarpine), output is automatically reduced. Whenever output is reduced (by pituitrin), intake of dilute food is promptly reduced. In the latter case it appears probable that the urge toward ingestion is stopped by the incipient accumulation of water in the body. The impairment of either ingestion or excretion will modify water turnover. Or, turnover is not regulated wholly, if at all, at the organs of exchange, but is regulated more nearly in accordance with the water content of the body. Ingestion and excretion are means of correcting a deficit or an excess, when they are not thrown out of action by an agent such as one of the substances here studied.

At present there seems to exist no evidence that rats or any other mammals regulate water content by the repeated correction of deficits, any more frequently than they regulate it by the correction of excesses. Exchanges by ingestion and by excretion are equally dependent upon other factors; they are intimate parts of a complex.

The methods here employed serve as assay procedures for substances that affect water exchange. By the method of Series I it is possible to assay atropine, pilocarpine or postpituitary hormones without any manipulative administration of water (by tube or needle). By the method of Series II any modifier of drinking may be evaluated; such modifiers may be termed antiposic agents. By the particular procedure of Series III, antidiuretic agents may be assayed under conditions of maximal water excretion.

Theories of 'thirst' have been mainly concerned with the localization of an effective excitation to drink. No agent has been found that *enhances* water intake without the intervention of bodily dehydration. The urge to drink has been *suppressed* appreciably by pilocarpine and doryl. Both these agents are regarded as cholinergic. It is not yet demonstrated that cholinergic substances in general inhibit drinking. The points of action of such substances are so numerous that no profitable speculations can at present indicate their locations. Local administrations of such substances may eventually help to define their points of action in the urge to drink.

#### SUMMARY

By diluting the food with large proportions of water, rats were induced to ingest large volumes of water which were continuously excreted. The large turnovers were inhibited by administering intramuscularly atropine, pilocarpine or postpituitary extracts. After rats had been without water for 48 hours, maximal ingestion was secured when water again became available. Ingestion and thirst were inhibited by

certain large doses of pilocarpine and of doryl. The inhibition was not found in all doses that stimulated excessive salivary flows. By administering water by stomach (6 doses, each 5% of the body wt.), water diuresis was induced. Of the agents tested, only postpituitary extracts appeared to be potent in suppressing the diuresis. Rapid turnovers of water in rats are regulated no more by output than by intake. Both exchanges are part of a complex which at present defies breakdown and which accords with the water content and (no doubt) other properties of the body.

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# PARTIAL NEPHRECTOMY AND THE WATER EXCHANGES OF RATS<sup>1</sup>

E. F. ADOLPH AND S. L. PARMINGTON

*From the Department of Physiology, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

WHEN rats ingested large quantities of water in obtaining food with which it was mixed (1), large volumes of urine were also excreted. This dietary regime provided an easy method by which to pass excess of water through the animals and hence made feasible a study of the consequences of excessive turnover of water.

The present investigation is a partial exploration of some adaptive mechanisms of water exchanges. The question arose, would ingestion be diminished if the excreting tissue were reduced? Further, does the amount of renal tissue determine or modify water diuresis? It is well known that partial nephrectomy leads to hypertrophy of remaining renal tissue. Does high turnover of water also influence the rate or amount of hypertrophy? Answers to these questions are given here.

## PROCEDURE

Each rat was maintained in a metabolism cage, in a cabinet kept at 27°C. In Series I the sole source of intake was a drinking cylinder containing 250 ml. of dilute milk. The milk mixture had 2.6 per cent of solids and was made each 24 hours by mixing 25 ml. of canned fortified milk (Formulac) with 0.5 gm. of benzoic acid and 225 ml. of distilled water. Of this liquid, rats drank quantities approximately equal to their own body weights each 24 hours (1).

Male rats of 150- to 200-gm. weight were given 3 to 10 days in which to become accustomed to the diet; then three days of control observations were allowed. On zero day rats were nephrectomized through flank incisions under ether anesthesia, in three diverse ways. In *Group A* a single kidney was excised (1 nephrectomized) after decapsulating it and tying the vessels of its hilus within the capsule. In *Group B* a half of one kidney was decapsulated and excised after tying a coarse thread through its middle; at the same time the other kidney was totally excised (1½ nephrectomized). In *Group C* half a kidney was excised alone, followed seven days later by excision of the remaining whole kidney (½ + 1 nephrectomized). In every case care was taken to leave intact the adrenals and their blood vessels. The period of the surgical operation deprived the rats of food and drink over several hours. Both the operated individuals of the three groups and the unoperated controls were returned to the same cages and diets as before. Subsequent intakes and urinary outputs were measured in 24-hour periods.

In Series II, male rats were tested, before and after 1½ nephrectomy, for excretory capacity. By forcing water into them through a stomach tube, the excretion of water attained a maximal rate, independently of the urges to drink. Warm distilled water equal to 5 per cent of the body weight was given at intervals of one-half hour for six or three administrations. Urine as spontaneously voided was collected in special small funnel-cages during at least six hours; its volume and the body weight were recorded every half hour. These animals were supplied *ad libitum* with dried whole milk and water.

Received for publication October 11, 1948.

<sup>1</sup> The investigation was aided by a contract between the Aeromedical Laboratory, U. S. Air Forces and the University of Rochester.



At surgical operation the excised renal tissue was placed in a tared weighing bottle. After the tissue's wet weight had been ascertained, it was dried in an oven at  $100^{\circ}\text{C}$ . for two or three days until a minimal weight was attained and checked. With certain exceptions each rat was killed at 7 to 18 days after operation, if it survived that long, and its remaining kidney was then treated similarly. These determinations furnished information as to the extent of hypertrophy and its composition.

*Daily Water Turnover (Series I).* Rats subjected to loss of  $1\frac{1}{2}$  kidneys at one operation (*Group B*) drank about one third of the usual amount of dilute food in the first 24-hour period (fig. 1). Correspondingly the output of urine was small. On subsequent days the intake gradually increased and, at the end of about seven days, stabilized at the rate of water turnover characteristic of the days previous to operation. Hence  $1\frac{1}{2}$  nephrectomy modified the intake and output of water, but

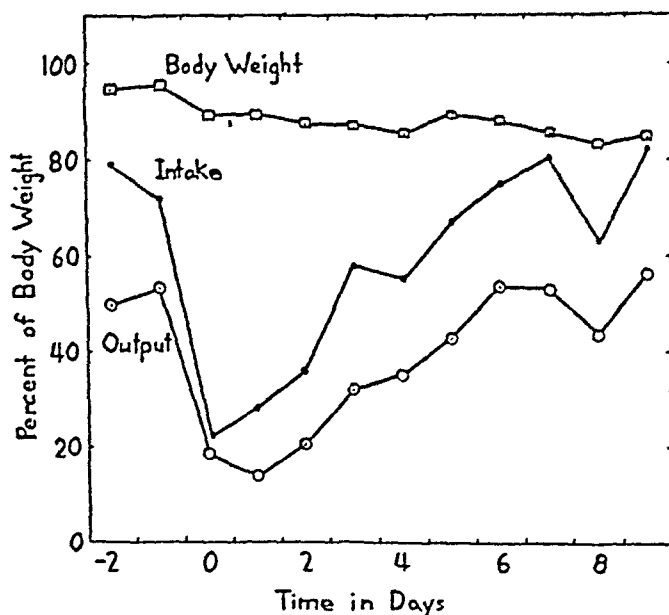


Fig. 1. MEAN BODY WT. and rates of fluid intake (2.6% milk solids) and of urinary output, in % of body wt/24hr., in 4 rats from which  $1\frac{1}{2}$  kidneys were excised on zero day.

only temporarily. The body weight progressively diminished during the days of observation, but no more rapidly than in unoperated rats on this dilute diet. As previously shown (1), maximal water intake prevailed only when the rats were furnished a mixture of milk which was so dilute (2.6% of solids) that maintenance of body weight failed by a slight margin.

Further results can be pictured in terms of fluid intakes, since urinary outputs paralleled them on each experiment. The disparities between fluid drunk and urine collected were due chiefly to incomplete catchment of urine, some of it evaporating before it ran down the funnel of the metabolism cage. It is seen (fig. 2) that excision of one kidney (*Group A*) diminished the intake of fluid but slightly and for only a single day at operation. Excision of half a kidney (*Group C*) likewise diminished the intake slightly for a single day; subsequent excision of the whole remaining kidney diminished it for two days. Unoperated controls showed a suggestion of diminution for a single day, due to the fact that they were denied food and drink for the duration of the surgical operation and recovery period in the other individuals.

In sum, removal at one operation of  $1\frac{1}{2}$  kidneys produced the only marked diminution of water turnover and that diminution lasted only six days.

The above experiments show that unilateral operations upon kidneys diminish water exchanges only on the day of operation. After bilateral operations on one day, six or seven days are required for recovery. Evidently the reduction of renal mass does not itself decrease the turnover of water (as in  $\frac{1}{2} + 1$  nephrectomized) but the immediate sequelae of operation do. It is well known (2, 3, 4) that excision of a kidney leads to inflammatory processes along with hyperplasia of proximal tubular epithelium in the other kidney. Much renal insufficiency temporarily prevailed, most of which effectively cleared up within seven days.

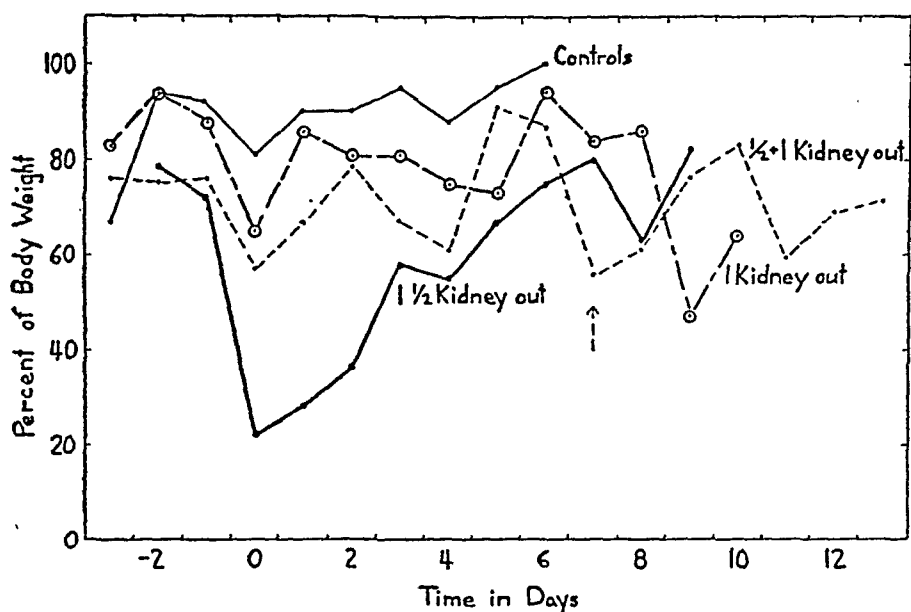


Fig. 2. MEAN RATES of fluid intake (2.6% milk solids) in % of body wt/24 hr., in rats subjected to 3 extents of nephrectomy upon zero day. Rats  $\frac{1}{2} + 1$  nephrectomized were also subjected to the second portion of nephrectomy upon the 7th day (at arrow). Each point is the mean of 12 in controls, 8 in  $\frac{1}{2} + 1$  nephrectomized, 4 in  $\frac{1}{2}$  and 4 in 1.

The severity of the operation was indicated by the fact that 43 rats underwent excision of  $1\frac{1}{2}$  kidneys in order that 16 could survive until planned autopsy was in order. The other 27 died at two to eight days following the operation, with signs of uremia. In them, it is believed, recovery of the reorganizing renal tissue was too slow to allow the rat to survive. In some, blood supply to the remaining renal tissue may have been occluded. Of 14 rats  $\frac{1}{2} + 1$  nephrectomized, 5 died, 3 of them after a single operation, and 2 after the second operation. Hypertension is known to develop gradually during the first two months after this operation (5, 6). Therefore the lethal effect seems to be only partly related to the amount of tissue excised; instead, it is related to the immediate tissue damage done at any one operation.

*Water Diuresis.* Control rats were tested repeatedly to find whether water excretion was more rapid after they had experience in water diuresis. The standard water administration (6 doses) was employed. Figure 3 shows that after several tests at semi-weekly intervals the rats excreted the water faster than in the first test.

As a result, the body weight did not reach so high an excess. Maximal rates of excretion seemed to be reached in the third test of each individual (table 1) and were not enhanced further in as many as eight repeated tests. These tests illustrated the fact that rats adapt to water excess by excreting the water faster, as was reported by Liling and Gaunt (7). In the present tests it was apparent that the faster excretion of water directly prevented the accumulation of so great an excess of water in the body and, consequently, avoided a part of the tendency for toxic effects of water excesses to occur. Most noteworthy is the fact that the water diuresis after adaptation was much more prompt in its onset and did not require the slow development which was characteristic of the first tests. The diuresis may have been reflexly in-

TABLE 1. MAXIMAL WATER LOADS AND MAXIMAL URINARY FLOWS IN RATS GIVEN BY STOMACH AT 30-MINUTE INTERVALS 6 DOSES OF WATER EQUAL TO 5 PER CENT OF BODY WEIGHT

SERIAL TEST	DAYS AFTER NEPHRECTOMY	NUMBER OF TESTS	MEAN MAXIMAL LOAD	ITS STANDARD ERROR	MEAN MAXIMAL FLOW	ITS STANDARD ERROR
			% of wt.		% of wt/hr.	
Unoperated						
1		25	12.5	$\pm 0.7$	6.0	$\pm 0.4$
2		8	9.2	$\pm 1.0$	7.0	$\pm 1.0$
3		5	8.0	$\pm 1.1$	8.8	$\pm 1.2$
4		5	9.4	$\pm 1.5$	8.6	$\pm 1.6$
5		5	9.7	$\pm 1.7$	7.2	$\pm 0.4$
6		3	9.5	$\pm 1.0$	8.0	$\pm 2.5$
$1\frac{1}{2}$ Nephrectomized						
1	2-3	8	19.7	$\pm 1.9$	3.0	$\pm 0.6$
2	4-6	7	17.3	$\pm 1.5$	4.4	$\pm 0.8$
3	7-10	7	19.1	$\pm 1.8$	5.0	$\pm 1.1$
4	11-17	7	16.7	$\pm 1.8$	3.8	$\pm 1.0$
5	18-24	7	15.5	$\pm 1.5$	3.8	$\pm 0.6$
6	25-31	6	17.5	$\pm 0.5$	5.0	$\pm 0.7$
7	32-38	4	16.8	$\pm 0.3$	5.4	$\pm 1.0$

hibited somewhat in the first and second tests by the procedure of repeated stomach-tubing. It may be remarked (fig. 3) that the most rapid urine flows were in the periods after the last administration of water; this is the case even in the tests after acclimatization to water administration. This phenomenon also suggests that there may have been a release from inhibition where manipulations of the rat ceased.

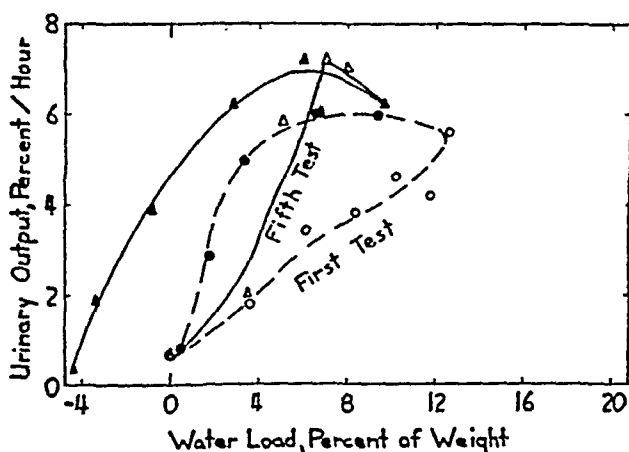
Individuals  $1\frac{1}{2}$  nephrectomized (Series II) were in all stages of adaptation, having had from 0 to 8 previous tests. After operation they were tested usually on the second, fifth and ninth days and subsequently at weekly intervals (table 1). Some individuals were too ill to be used for test; these died. In addition, those with the lowest rates of excretion during water tests soon died before a second test could be made. There was no evidence that the water administrations as such prevented survival.

In first water tests after  $1\frac{1}{2}$  nephrectomy (fig. 4), excretion of water was much

slower than before operation (about  $\frac{1}{4}$  as rapid) and water accumulated to an average of 20 per cent of the body weight. Subsequently the rate and promptness of diuresis increased, so that in the fifth tests (at 18–24 days postoperatively) the rates were about one-half and, later, two-thirds of those characteristic of the same individuals before partial nephrectomy. The higher rates of excretion then prevented so great an accumulation of water in the body.

Apparently one fourth of the renal tissue could on the average excrete urine one-half or two-thirds as fast as all the renal tissue. Individual tests showed as much as four fifths of the mean excretion manifested by intact kidneys. Four rats with  $1\frac{1}{2}$  kidneys excised were kept for  $1\frac{1}{2}$  to 3 months and successive tests tended to show progressive recovery of maximal urinary flow. The results suggested that the limit of functional recovery was then approximately reached. It may be concluded that while complete absence of renal tissue would, of course, prevent water excretion, less

Fig. 3. URINARY OUTPUT in relation to water load in intact rats. First test, mean of 25 individuals; 5th test, mean of 5 individuals. In each test water equal to 5% of the body wt. was placed in the stomach each half hour for 6 doses. Points represent the mean water retention and mean rate of excretion during each half hour period; open points during increasing water loads, solid points during subsequent decreasing loads.



than one half of the tissue is sufficient for a maximal water diuresis at rates approaching the rates of excretion possible in intact kidneys.

The increase in rate of water excretion that followed recovery from partial nephrectomy was not appreciably due to the above adaptation process in the water test. The increase was about as great in previously adapted rats as in fresh ones. Presumably it was chiefly concerned with reorganization of functions in the remaining renal tissue. It was apparent up to 24 days after operation, which was a much longer period of increase than was visible in the drinking tests (fig. 1). Evidently the turnover of fluid ingested in the form of 2.6 per cent milk solids was limited by the maximal rate of water excretion only up to the seventh postoperative day; thereafter about half the maximal rate characteristic of the intact rat prevailed and was sufficient to maintain the turnover. Nevertheless, to maintain that turnover the maximal rate (3%/hr.) had to be approximately maintained by the rat in every hour of the day and night.

It might be supposed that the large excesses of body water forced upon the rats in six doses of 5 per cent of the body weight were too large for optimal excretory functioning. Other tests therefore were done in which three doses of 5 per cent each were given (fig. 5). In control rats the maximal rates of excretion attained were 5 per cent/hr., instead of the 6 to 8 per cent/hr. after six doses. Two days after  $1\frac{1}{2}$

nephrectomy, maximal diuresis was about 3 per cent/hr., which was very close to the mean rate found after six doses (fig. 4). The total effect of nephrectomy upon water diuresis was therefore manifested to about the same degree whether three or six doses of water were administered and whether maximal water loads were 20 per cent or only 10 per cent of the body weight.

Current views as to how water is excreted in mammalian kidneys emphasize the enormous rates of believed capsular filtration. According to these views, reduced renal tissue would have no difficulty in forming urine rapidly. The estimated rates of filtration (maximal creatinine clearances) in normal rats are reported as 90 per cent of the body wt/hr. (8). Unless the filtration rate increases in the residual fraction of renal tissue, as is altogether likely, the remaining one fourth of kidney substance was during the water test excreting urine equal to one fifth of its original filtra-

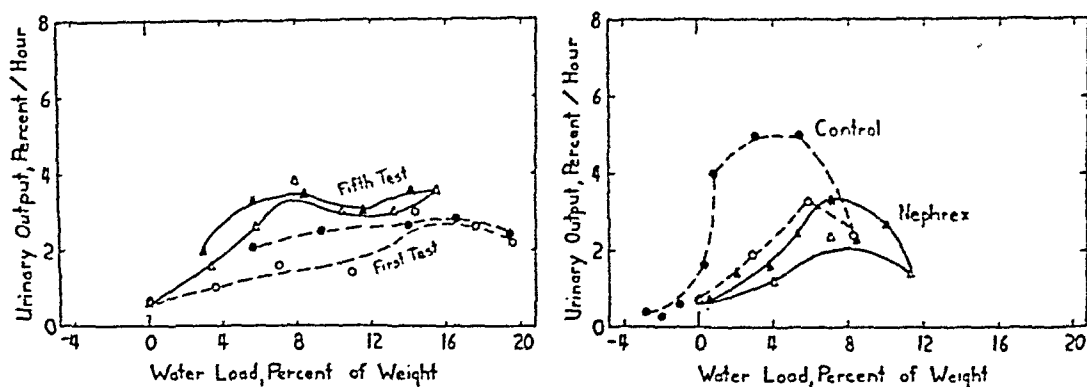


Fig. 4 (left). URINARY OUTPUT in relation to water load in nephrectomized rats. Procedure same as in fig. 3. First tests (8) were done 2 or 3 days after  $1\frac{1}{2}$  nephrectomy; 5th tests (7) were 18 to 24 days after operation.

Fig. 5 (right). URINARY OUTPUT in relation to water load in rats given 3 doses of 5% of wt. of water; otherwise procedure was same as in fig. 3. Control tests (11) were before operation; other tests (4) were 2 to 3 days after  $1\frac{1}{2}$  nephrectomy.

tion flow (22%/ hr.). In anesthetized rabbits under sulfate diuresis it is estimated that urine flow may equal two thirds of the capsular filtration rates and, under bi-chromate nephritis, may even equal them (9). Solutes that are ordinarily 'reabsorbed' appeared in the urine under those conditions. In the rats studied here, neither glucose nor protein appeared in the diuretic urine more frequently than in ordinary urine.

The experiments show clearly that the maximal rate of water excretion in the normal rat is not wholly limited by the amount of renal tissue. The flow or pressure of the blood may be limiting or the rate of water absorption from the intestine may be limiting. The latter would presumably be unaffected by partial nephrectomy, and if limiting, would not lead to the convulsions which occur; the former may adjust within a brief period of time after operation.

The ordinary rates of water turnover by rats as a result of  $1\frac{1}{2}$  nephrectomy remain to be mentioned. Five individuals were intensively studied for 3 days before operation and 17 days after it. Food intakes were less for 3 days after operation, but water intakes were less only upon the day of operation and urinary output paral-

leled them. Hence, though others have observed polyuria (5, 10), we found no important or significant change in metabolism of solids or of water beyond the three days after nephrectomy. Whether dietary or other factors are responsible for the difference is not clear.

*Water Intoxication.* Convulsions were induced when large quantities of water were retained in rats, as in other mammals. In the present series, convulsions were absent in all but one of 54 tests before nephrectomy (25 individuals). They occurred in 14 of 54 tests after nephrectomy (14 individuals). Any one individual might convulse in some tests and not in others. Somewhat more water was retained in the nephrectomized individuals (16–20% of the body wt. at 3 hr. of the test) as compared with the intact rats (10–13%). At 16 to 22 per cent retention lies the usual threshold for convulsions in rats. It should also be recognized that other factors than water content or dilution of body fluids enter into the induction of convulsions, since marked species differences prevail.

Hemoglobin sometimes appeared in the urine during water tests, with equal frequency in the controls and in the nephrectomized. Hence hemoglobinuria did not result from the state of the kidneys nor from the extent of the water excesses present in the body as a whole; probably it was produced by the dilution of blood during the process of water absorption from the intestine. Aside from the days of water tests, blood and protein appeared in the urine almost exclusively on the days of surgical operations and were evidences of local damage.

*Renal Hypertrophy.* It has frequently been supposed that prolonged exaggerations of water turnover lead to renal hypertrophy. As evidence for this, the large kidneys sometimes found at autopsies of habitual beer-drinkers are cited. Rats that received only 2.6 per cent of milk solids in water were often excreting their body weights of urine every day. Some 25 of them (not nephrectomized) were killed, their kidneys were weighed and compared on the basis of body weight with the standard series reported by others (11). No appreciable difference was found (table 2). It is concluded that rapid turnover of water is not a stimulus to hypertrophy of the kidneys.

Evidence of renal hypertrophy inevitably involves a knowledge of changes of body weight and of renal growth that occur during any dietary regime. Upon the above dilute-food regime the rats were slowly losing weight. It might be that the kidneys did not grow smaller as the body shrank and that the body weight characteristic of the day upon which the regime started should be used for prediction of kidney weights. After certain types of weight diminution due to dietary restriction, however, it has been ascertained that the kidneys diminish proportionally in weight (12). Actually the body weight diminutions were sufficiently small (table 2) so that the hypertrophy would not be significantly different if the initial body weights had been used in predicting renal weights.

Partial nephrectomy is well known to induce hypertrophy of the remaining renal tissue. Would this hypertrophy be greater in rats having high turnovers of water? The extent of hypertrophy was accurately judged in the 1-nephrectomized rats by comparing the weight of the kidney obtained at autopsy with the weight of the kidney first excised at operation. The hypertrophy was moderate (table 2) and of the same

magnitude as in adult 1-nephrectomized rats maintained upon diets of low water content but of about the same protein contents (13, 14).

After  $1\frac{1}{2}$  kidneys had been removed, the hypertrophy could be estimated only by adding the weight of the half kidney removed at operation to the weight of tissue found at autopsy. The average percentage hypertrophies were 15 per cent in the rats operated in two stages and 30 per cent in the rats operated bilaterally at one

TABLE 2. MEAN WEIGHTS AND WATER CONTENTS (WITH STANDARD ERRORS) OF THE KIDNEYS OF RATS UPON DIVERSE REGIMES

REGIME	NO. OF ANIMALS	MAX. BODY WT.	BODY WT. AT NEPHRECTOMY	WT. OF ONE KIDNEY,	WATER IN TISSUE	HALF KIDNEY WT.	WATER IN TISSUE	REMAINING KIDNEY WT. AT AUTOPSY	WATER IN TISSUE	RENAL HYPERTROPHY
			gm.	gm.	%	gm.	%	gm.	%	%
Dry milk diet, controls	7	186.5	180.3	0.750	76.0 $\pm 1.22$					
Dry milk diet, $1\frac{1}{2}$ kidneys out	7	234.9	228.6	0.893	75.2 $\pm 1.47$	0.386	76.3 $\pm 0.84$			
	11 <sup>1</sup>	206.4	198.4	0.844	75.8 $\pm 1.67$	0.400	78.7 $\pm 0.56$			
2.6% milk, controls	25	173.5	153.6	0.729	76.7 $\pm 0.57$					
2.6% milk, 1 kidney out	9	178.0	164.5	0.723	76.7 $\pm 1.88$			0.788 (9)	75.2 $\pm 1.20$	13.1
2.6% milk, ( $\frac{1}{2}$ + 1) kidneys out	8	185.3	166.4	0.707	75.6 $\pm 0.82$	0.308	77.5 $\pm 0.59$	0.499 (8)	78.1 $\pm 0.81$	14.6
	2 <sup>1</sup>	158.5	127	0.578	78.8	0.225	77.3			
2.6% milk, $1\frac{1}{2}$ kidneys out	9	202.7	189.1	0.820	77.0 $\pm 1.31$	0.344	76.7 $\pm 2.60$	0.725 (8)	76.7 $\pm 0.17$	29.6
	16 <sup>1</sup>	197.3	178.3	0.766	76.9 $\pm 0.49$	0.339	77.2 $\pm 1.68$			

<sup>1</sup> Rats died prematurely.

etherization. Previous investigators (5) carried out 75 to 85 per cent excision of kidneys in rats but did not attempt to estimate the resulting hypertrophies.

It is plain that after partial nephrectomy, the remaining renal tissue undergoes no more hypertrophy with the stimulus of rapid turnover of water than without it. A hypertrophy of 30 per cent by weight is insufficient, in fact, to account for the amount of function manifested during water diuresis and it can be concluded that water diuresis was not greatly limited by the amount of renal tissue. It is probable that more extensive hypertrophy occurs in certain proximal tubular structures of the kidneys, as pictured by Oliver (4) and Rollason (15), but not in the number of nephra (16, 17).

The water contents of excised renal tissues were ascertained (table 2). On logarithmic grids the lines drawn through the points of absolute water contents are parallel to those drawn through the points of absolute kidney weights. Hence the relative water contents were not different before and after hypertrophy, as the tabulated means show. In hypertrophied kidneys some investigators have reported that the water fraction is increased (14); others that it is unchanged (3, 21).

Slight increases in relative water content were reported in kidneys taken from rats killed while they had water diuresis (18). It is possible that direct comparisons between our animals in high water turnover and those consuming dry food would have demonstrated a barely significant increment of water in the renal tissue.

#### COMMENT

The rate of forced water ingestion was reduced for a few days following excision of more than one kidney. Evidently the excretion of water was then retarded, leading to an immediate inhibition of intake when only dilute food was available. As the reduced renal tissue recovered from the operation, the intake was restored to its initial rate. This inhibition constitutes evidence that a slight accumulation of excessive water in the body is sufficient to discourage intake of water, even though the urge to obtain food is somewhat increased at the same time. Some day it will be known just how an increase of body water content may suppress the urge to drink.

The rate of water excretion, it is shown, depends upon the amount of functioning renal tissue to only a limited extent; it is further limited temporarily when the functioning is upset as a sequel of surgical manipulation. Once the renal tissue has recovered from the immediate effects of operation, one fourth of the renal bulk is quite sufficient to carry on either maximal forced turnover of water or half-maximal forced water diuresis. While a marked reduction of renal bulk could limit water diuresis, another correlative of functional capacity than mere bulk of tissue may some day be identified.

Maximal rates of urine production have now been obtained in normal rats after adaptation to repeated administrations of water. These rates average 8 per cent of the body wt./hr. The shape of the curve, relating water excess to rate of excretion (fig. 3), is sensibly linear up to 4 per cent of the wt./hr.; at higher rates it tends to be independent of water load. Hence there is reason to believe that still higher loads (above 15% of the wt.) will not induce faster diuresis. Loads of 20 per cent usually induce convulsions in rats; these convulsions are not lethal, we find. The occurrence of convulsions is not correlated with the period of time since nephrectomy and hence not with the amount of arterial hypertension.

An intermediary regulator of renal hypertrophy is believed to be the anterior hypophysis. When it has been removed, unilateral nephrectomy no longer leads to renal hypertrophy (19); instead, the kidneys diminish in size and possibly in function (20). The chief factors that exaggerate the hypertrophy after partial nephrectomy in rats are: youth, high protein intake and testosterone administration (21). All are believed to represent a high intensity of protein metabolism, which might in turn excite the intermediaries. These same factors accelerate the enlargement of the kidneys without nephrectomy. To many other factors that have been found not to



induce renal enlargement, continuous rapid water excretion may now be added. The present results confirm in this respect the negative results obtained in the attempts of Hinman and Belt (22) and Chanutin and Ludewig (23) to influence renal hypertrophy or water retention by injecting considerable volumes of saline or urea solution each day.

#### SUMMARY

After three to five repetitions of excessive water administration in normal rats, water diuresis was characterized by less delay in onset and greater rates of output. Maximal water diuresis was greatly diminished when  $1\frac{1}{2}$  kidneys were excised. Within four weeks after operation water diuresis recovered to be, on the average, two thirds as rapid as before it. Water ingestion, that was forced by the method of furnishing only dilute food, was diminished only for one week following  $1\frac{1}{2}$  nephrectomy. Half or one nephrectomy did not have any appreciable effect upon water ingestion. Following  $1\frac{1}{2}$  nephrectomy, the hypertrophy of the remaining renal tissue was no greater upon the regime of forced water than without it. Evidently prolonged excessive excretion of water was not a factor in determining the amount of hypertrophy. The water content of the kidneys was not significantly increased by the forced water regime nor by the hypertrophy superimposed upon it.

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# MYOGRAPHIC STUDY OF THE CAT'S HEART: EFFECT OF CHANGES IN VENOUS RETURN AND IN PERIPHERAL RESISTANCE ON VENTRICULAR CONTRACTION

JOSEPH R. DiPALMA AND RICHARD A. REISS<sup>1</sup>

*From the Department of Physiology and Pharmacology, Long Island College of Medicine*

BROOKLYN, NEW YORK

**M**YOGRAPHIC studies of ventricular contraction have been used to demonstrate that under certain conditions the heart contracts more or less forcefully (1, 2). For the most part it has been overlooked that the heart resembles most closely an elastic hollow sphere as first pointed out by Bayliss and later by Gesell (3, 4). The fact that the muscle fibers of the heart travel in different directions not only on the surface but in the depth of the myocardium further complicates matters. Moreover, with dilation the heart wall becomes thinner and the muscle fibers change their relationship to each other; most fibers are lengthened some conceivably are shortened. Finally the tension in the different layers of the heart wall is not equal in all layers. Particularly in the left ventricle it has been shown that the tension is greatest near the endocardium gradually diminishing towards the epicardium (5). With changes in heart size this gradient of tension is altered as the wall becomes thicker or thinner and this might be reflected in myographic measurements.

## METHODS

In all the experiments cats (8 female, 7 male) weighing between 2 and 5 kg. were used. Anesthesia was intraperitoneal dial. The chests were opened and artificial respiration was given by means of an automatic pump. The venous pressure measurements were made by direct cannulation using a saline manometer. Heparin in the saline kept the cannulae free of clots. Arterial pressure was recorded from the carotid artery by use of a Hürthle Manometer in most of the cats and by a Hamilton Manometer with a cannula in the aorta in the others. The contractile force of both the right and left ventricular muscle was recorded by three different myographic techniques in separate experiments. Only the second method will be discussed in detail since the others have previously been described.

**METHOD I. *Cushny's Weighted Myograph.*** Essentially the same apparatus was used and techniques followed as described by Walton and Brodie (1). This consisted of an ordinary Cushny's myograph to which was added a calibrated steel spring. In practice by increasing the tension on the spring the movable lever was weighted to the exact point where motion of muscle no longer occurred. This point was called the isometric systolic tension (I.S.T.). In our experiments the myographs were sewn to both the right and left ventricles. The one for the right ventricle was placed on the anterior surface of the heart; the direction of contraction was towards the right shoulder region. The left Cushny myograph was placed on the left lateral wall of the heart and in this in-

Received for publication September 22, 1948.

<sup>1</sup> Research Fellow in Physiology.

stance the direction of contraction was parallel to the longitudinal axis of the cat. In all instances the movable lever of the myocardiograph was attached as far as practicable from the apex of the heart.

**METHOD II. *Dynamograph.*** The objection frequently raised with the Cushny type of myograph is that with changes in heart size the lever arms measure different areas of muscle contraction. To overcome this difficulty a new type of myograph was designed (fig. 1). A lucite capsule whose lower surface enclosed an area of exactly one square centimeter was applied to the anterior surface of the right ventricle by means of suitable clamps. In the center of the circular aperture of the capsule a silk ligature was sewn to the heart wall. The upper end of the thread was tied to an isometric spring which was made of 31 turns of B&S 20-gauge piano wire in a  $\frac{3}{8}$ " diameter closely wound spiral. Another length of thread extended from the lower end of the spring over a pulley either to a lever system for kymograph recording or a mirror for optical recording. It can be seen how the myograph works by referring to figure 1. During diastole when there is no tension on the wall of the heart the muscle bulges into the capsule. In systole the heart wall becomes tense, assumes a spherical shape and resists distortion of its surface hence little muscle can be pulled into the capsule. It is this in-and-out motion which is recorded. The spring and recording system is calibrated for each experiment by hanging weights on the lower end. Once set up the desired initial tension can be secured by raising or lowering the spring. In practice it was found that 10 gm. was the least initial tension compatible with smooth recording. The heart muscle remained well nourished and no local cyanosis was noted in five consecutive experiments. The typical records obtained with this device will be discussed in connection with the results. It should be obvious that the main advantage of this myograph is that the force of contraction of one square centimeter of heart muscle is measured despite changes in contour or size of the heart.

**METHOD III. *Intramycardia Pressure.*** This method has been extensively described before and consists of recording optically the changes in tension of an artery segment imbedded in the wall of the left ventricle (5). Although this method has been criticized by Gregg the findings in this study are limited to comparative values in the same animal and for this purpose are reliable (6).

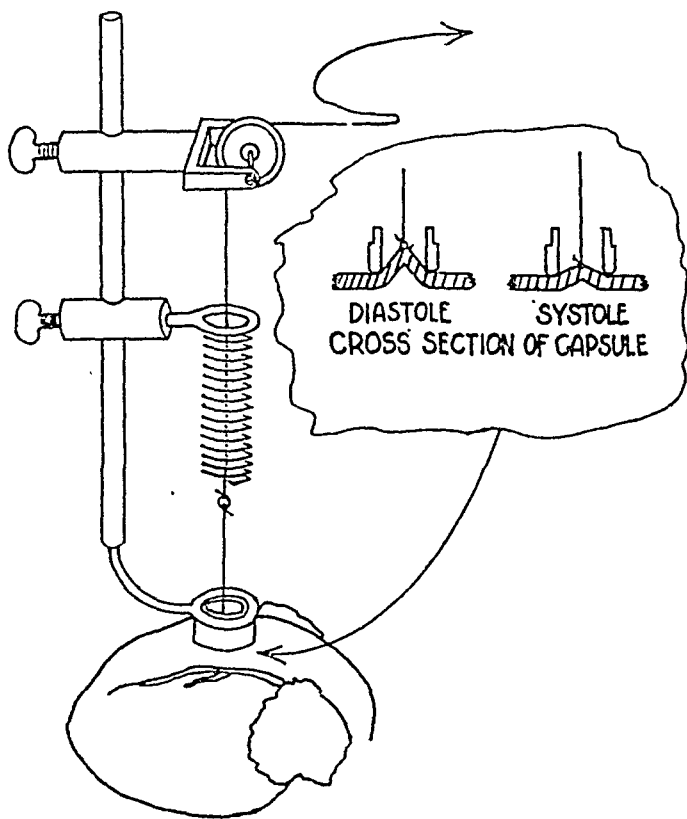
## RESULTS

***Unweighted Cushny Myograph.*** When 30 cc. of N saline is infused at the rate of 10 cc/min. the degree of excursion of the segments of muscle under study in both the right and left ventricle decreases (fig. 2). This occurs despite the rise in arterial pressure. It does not occur if the infusion is small or the animal is dehydrated, i.e. enough fluid must be given to increase the size of the heart appreciably. A decreased venous return caused by clamping the inferior vena cava results in increased excursion of the heart muscle despite a fall in arterial pressure (fig. 3). Again this result will not be obtained if the decreased venous return does not cause a decrease in cardiac size, i.e. if the heart is at a minimum size initially. These results duplicated in four separate experiments show that an increase in cardiac diameter is associated with decreased excursion of the muscle fiber and a decreased diameter with increased excursion.

***Isometric Systolic Tension.*** Appropriate weighting of the Cushny myograph as in the technique of Walton and Brodie (1) permitted a determination of the isometric systolic tension (I.S.T.). These results obtained in one of three typical experiments are summarized in tables 1 and 2. With increased venous return the I.S.T. falls from a control of 18 gm. in the right ventricle to a low of 11 gm. in 2 minutes, recovery taking place in 15 minutes. During this period there is a rise in venous pressure and in arterial pressure (table 1). Changes of the same order occur in the left ventricle. Similar measurements during decreased venous return (clamping

the inferior vena cava) show the opposite result. In this case the clamping was done at intervals so as to avoid anoxia of the heart muscle. Table 2 shows that there is a slight but definite rise in I.S.T. when the return of blood to the heart is decreased, more marked in the right ventricle as compared to the left. The reason for this is felt to be because of the difficulty of properly applying the myograph to the posteriorly placed left ventricle rather than to actual differences in response of the two ventricles. This occurs despite a marked fall in arterial pressure and venous pressure. Again it is inferred that the major cause for these observed changes in I.S.T. is the change in cardiac diameter.

Fig. 1. DYNAMOGRAPH. A special type of myograph which permits measurement of contractility of 1 sq. cm. of intact heart muscle, in spite of changes in cardiac diameter.



*Dynamograph Records.* This instrument gave relative measurement of the force of contraction of one square centimeter of muscle on the surface of the right ventricle, in spite of changes in cardiac size. Figure 4 shows the results obtained in one of five typical experiments. Starting on the left is the control period where the tension is 30 gm/sq. cm. diastolic (load tension) and rises to 68 gm/sq. cm. in systole. The arterial pressure is 65 mm. Hg. With an infusion of 30 cc. N saline, 10 cc/min. the diastolic tension has risen to 37 gm/sq. cm. and the systolic tension has fallen to 60 gm/sq. cm. at the end of 30 seconds. Hence the force developed during contraction has changed from 38 gm/sq. cm. in the control period to 23 gm/sq. cm. during venous infusion. Again this occurs in spite of arterial pressure rise. Similar changes occur in tension but in the opposite direction when the venous return is decreased by clamping the inferior vena cava (fig. 4). Thus this method gave simi-

lar qualitative results as the weighted Cushny myograph but under conditions where the surface area of the muscle under test was kept constant.

*Intramyocardial Pressure.* It was felt desirable to check the paradoxical results obtained with the Cushny myograph and the dynamograph with a different method of recording. Figure 5, *A* and *B*, shows the result obtained in a typical experiment with the infusion of 35 cc. of N saline at the rate of 10 cc/min. Control arterial pressure was 87/68 mm. Hg (fig. 5 *B*). At the termination of the infusion the pressures were 97/62 and 78 mm. Hg respectively (not shown in fig. 5). In this experiment the carotid artery segment was located just to the left of the ramus descendens anterior artery and at one-third the depth of the left ventricular wall measuring from the epicardial surface. Thus this experiment permits the conclusion that the lateral tension developed during systole in the wall of the left ventricle decreases with increases in cardiac diameter brought about by augmented venous return.

*Initial Tension and Force of Contraction of Ventricular Muscle.* The results thus far conclusively demonstrate that increases in venous return with the consequent increase in cardiac diameter (initial length) are attended by a decreased ability of the myocardial muscle to shorten (as measured by these techniques). The question may well be asked what does serve as a stimulus for augmenting myocardial contractility? The dynamograph is a suitable instrument to test the effect of increased initial tension on the force of contraction. Figure 6 shows one such experiment. Arterial pressure remained relatively constant at a mean of 90 mm. Hg so it may be assumed that the state of the myocardium remained constant throughout the experiment. When the dynamograph diastolic tension was set at 10 gm/sq. cm. the systolic tension developed was 40 gm/sq. cm. Increases in diastolic tension to 27 and to 40 gm/sq. cm. resulted in further rises in systolic tension of 75 and 100 respectively. There is no doubt that an increased initial tension causes enhanced cardiac muscle contraction.

*Intraventricular Tension and Force of Contraction.* Clamping the aorta or pulmonary artery provides a convenient means of raising the intraventricular tension or initial tension. In figure 5, *C* and *D*, the effects of clamping the aorta on intramyocardial pressure are shown. In figure 5 *C*, the control blood pressure was 93/70 mm. Hg and the intramyocardial pressure 80 mm. Hg. Clamping the aorta raised the intramyocardial pressure to 165 mm. Hg. Similar qualitative results were obtained using the dynamograph and the weighted Cushny myograph on the right ventricle but these are not included for the sake of brevity. It must be pointed out that raising the initial tension in this manner also increases the initial length as the heart regularly dilates with this procedure. However it has been demonstrated above that increases in initial length do not augment the force of cardiac contraction (measured with the myographs) and these last experiments indicate the importance of initial tension.

#### DISCUSSION

The perfect myograph would permit mensuration of the force of contraction of a constant volume of heart muscle despite changes in heart radius. Such an instrument has not been devised. The dynamograph used in this study while capable of

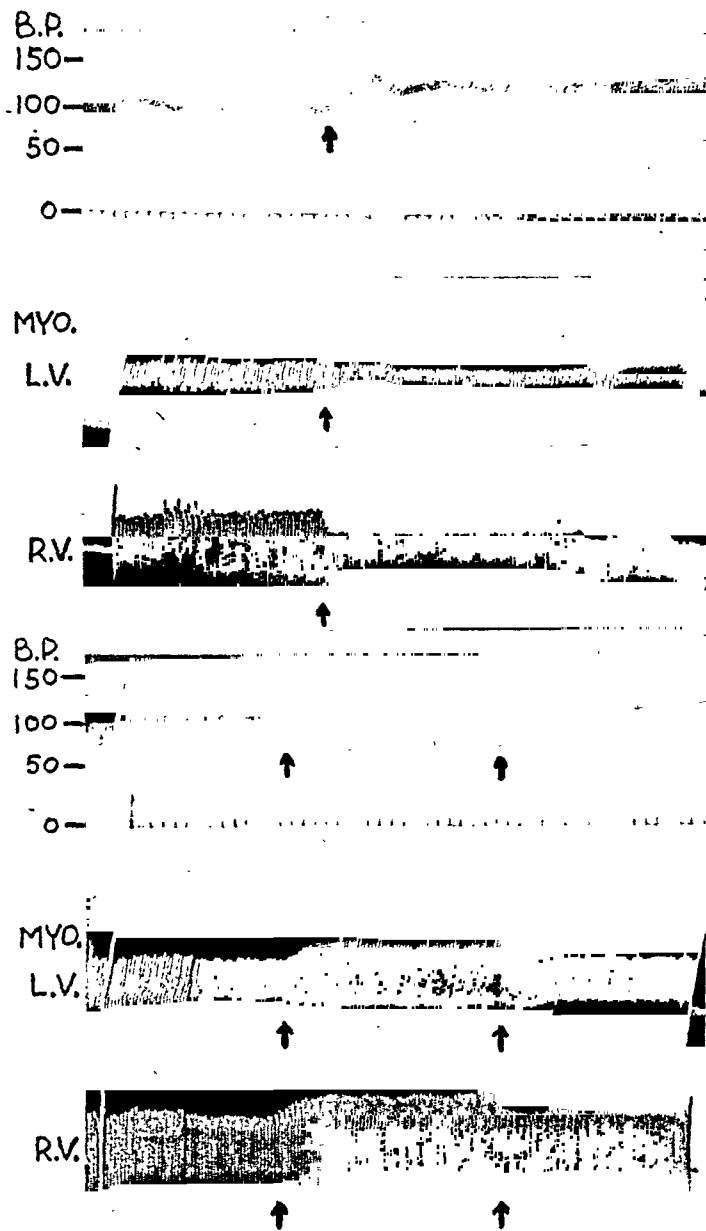
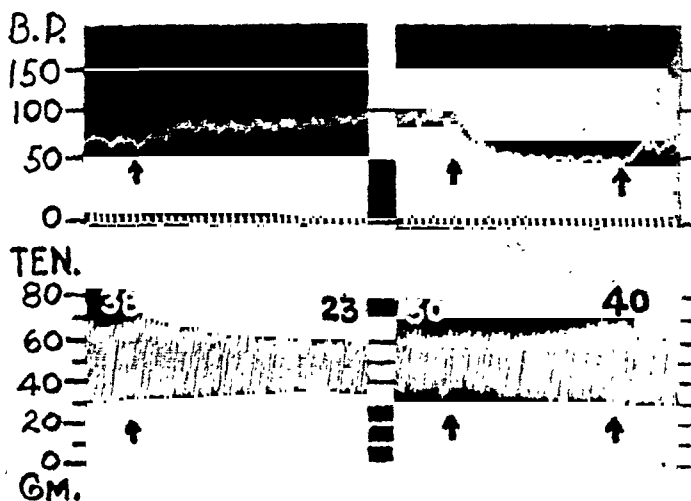


Fig. 2( upper). INCREASED VENOUS RETURN, unweighted Cushny myograph. From above downwards, arterial pressure, myograph left ventricle and right ventricle. Note the decrease in excursion with the onset of increased venous return (arrows).

Fig. 3 (lower). DECREASED VENOUS RETURN, unweighted Cushny myograph. Tracings as in fig. 2. Note the increase in excursion with the onset of decreased venous return brought about by clamping the inferior vena cava (arrows).

Fig. 4. DYNAMOGRAPH RECORDS. Top, blood pressure. Bottom, tension of 1 sq. cm. of right ventricle. Left, note that with increased venous return the tension decreases from 38 (68 minus 30) to 23 (60 minus 37) gm./sq. cm. Right, the opposite changes occur with decreased venous return.



measuring a constant area of muscle still does not account for changes in thickness of the heart wall. Cushny's myograph suffers from the added disadvantage that the distance between the recording arms changes as the diameter of the heart is

Fig. 5. INTRAMYOCARDIAL PRESSURE measured from an imbedded carotid artery segment in the wall of the left ventricle (see text) related to aortic blood pressure. *A*, control period; *B*, during increased venous return. Note that the lateral tension in the left ventricle decreases from 90 (290 minus 200) to 60 (260 minus 200) mm. Hg in spite of a constant aortic arterial pressure. Note also that while heart rate is constant the systolic period increased. Cardiac diastolic diameter had increased. *C*, another control period; *D*, during clamping of the aorta distal to the innominate artery. Note in this case the great increase in lateral tension.

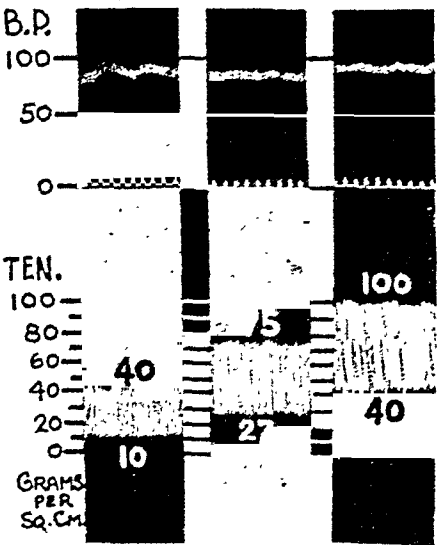
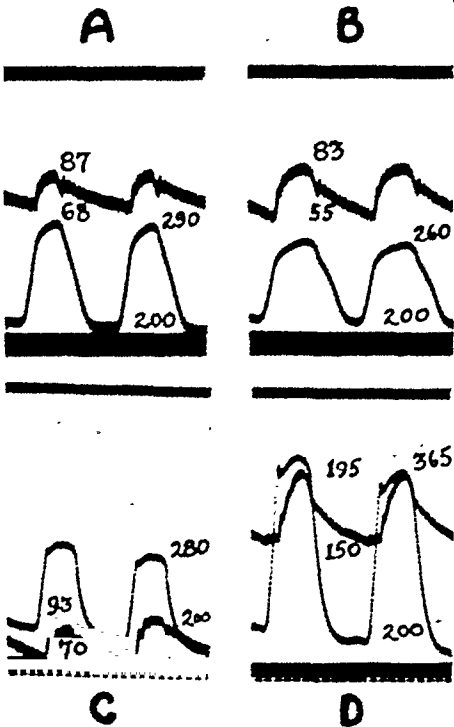


Fig. 6. DEMONSTRATION that an increase in initial tension causes increased contractility of the intact right ventricle. *Top*, arterial pressure; *bottom*, dynamograph. Note that with increases in initial tension from 10 to 27 to 40 gm./sq. cm. the maximum systolic tension developed is 40, 75 and 100 gm./sq. cm. respectively. Arterial pressure has remained constant throughout.

altered. An imbedded artery segment also obviously does not have a constant volume of muscle acting upon it under conditions where the heart wall varies in thickness. These considerations preclude using the myograph in any quantitative study of heart muscle contractility.

TABLE 1

TIME	ISOMETRIC SYSTOLIC TENSION, GM.		CM. H <sub>2</sub> O VENOUS PP.	MM. Hg ARTERIAL PP.
	R.V.	L.V.		
Control o	18	30	2.1	50
<i>Infusion 25 cc. saline, 10 cc. min.</i>				
sec. 10	12	23	2.3	60
20	11	22	2.5	65
40			3.0	70
60	11.5	22	3.2	70
80	12	23	3.4	73
180	11	22	3.6	80
min. 9	14	24	3.0	78
15	16	29	2.5	75

R.V. = Right ventricle. L.V. = Left ventricle.

TABLE 2

TIME, MIN.	ISOMETRIC SYSTOLIC TENSION, GM.		CM. H <sub>2</sub> O VENOUS PP.	MM. Hg ARTERIAL PP.
	R.V.	L.V.		
<i>Controls</i>				
0	15	36	2.7	73
2	17	40	2.5	73
4	16	32	2.2	74
6	17	31	2.3	75
Average.....	16.2	34.7	2.4	74
<i>During decreased venous return</i>				
8	18	33	1.5	10
10	21	34	1.3	12
12	23	37	1.3	15
14	24	39	1.8	25
Average.....	21.5	35.7	1.5	15.5
<i>Controls</i>				
9	18	30	2.8	80
11	16	27	2.4	85
13	16	28	3.0	75
Average.....	16.6	28.3	2.7	80

R.V. = Right ventricle. L.V. = Left ventricle.

Qualitative deductions may be permitted from myographic study provided certain physical relationships of the heart are kept in mind. The heart is a hollow globular



organ most closely resembling a hollow elastic sphere such as a soap bubble (3, 4). When the resting or diastolic diameter of such an organ increases, the volume contents increase as the radius cubed divided by three while the surface area only as the radius squared. To now expel its contents the muscle need not shorten through as great a distance (4). Our results show that this is true for the cat's heart under conditions of increasing venous return (figs. 2 and 3). The total volume of muscle composing the wall of a ventricle cannot change acutely. With an increase in cardiac radius the wall must perforce become thinner since it is spread over a greater surface area. This has the effect of showing a decreased contractility when studied myographically. The reason for this is obviously the fact that with the increase in cardiac radius a relatively smaller amount of muscle is being measured by the myograph. Our results conform perfectly to this interpretation (tables 1 and 2, figs. 4 and 5).

On the other hand, when the initial tension is altered either locally or by increasing peripheral resistance our results show uniformly that the ability of the heart muscle to shorten is enhanced (figs. 5 and 6). This result obtains even when the radius of the heart is increased because of the increased load of a greater peripheral resistance. This would seem to indicate that changes in initial tension are capable of inducing a positive inotropic effect on the muscle fiber. In contrast changes in initial length have per se no effect on the state of contractility itself. Heart performance is altered merely because of the physical peculiarity of a hollow sphere, i.e. volume/surface area ratio is changed so that the heart wall has a better or worse mechanical advantage.

Finally it should be pointed out that any type of myographic study of heart muscle should take cognizance of the results of such experiments as these. For example the comparison of different drugs on myocardial contractility by a myographic technique should be attended by observations of cardiac diameter and of changes in peripheral resistance. It is obvious that if a drug has the effect of increasing cardiac radius it will also have the apparent result of decreasing contractility. Also a drug which increases peripheral resistance will raise the initial tension and the myograph will record an increase in contractility which may not be a direct myocardial effect.

#### SUMMARY

Using three different myographic techniques the nature of cardiac contraction was studied during increased and decreased venous return and clamping of the aorta. Cats with the chest opened but with intact circulations were used. Analysis of the results was made on the basis that the heart resembles most closely an elastic hollow sphere. The results justify the following conclusions: 1) During increased venous return a segment of heart muscle under study contracts through less distance. The opposite is true for decreased venous return. 2) During increased venous return a segment of heart muscle contracts less forcibly. The opposite is true for decreased venous return. 3) During clamping of the aorta and also with increases in initial tension induced locally with a special myograph (dynamograph) a segment of heart

muscle contracts more forcibly. Therefore, increases in initial tension cause the heart muscle fibers to contract more forcibly.

These results would seem to indicate that the ability of the heart muscle fiber to shorten is not altered by changes in initial length but is altered by changes in initial tension. In the former instance work output is changed merely by a shift in the volume/surface area ratio of heart contents to heart wall. In the latter instance work performance is increased or decreased by an actual change in ability of the muscle fiber to shorten.

The artifacts and mechanical paradoxes of myographic recording were pointed out and discussed. Qualitative deductions are permitted only when concomitant changes in heart radius and peripheral resistance are taken into account.

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# RIGHT AND LEFT HEART FAILURE: UNILATERAL RISES IN RIGHT AND LEFT AURICULAR PRESSURE IN HYPERVOLEMIC CATS FOLLOWING NEAR LETHAL DOSES OF QUINIDINE, AURICULAR FIBRILLATION AND EPINEPHRINE

RICHARD A. REISS<sup>1</sup> AND JOSEPH R. DiPALMA

*From the Department of Physiology and Pharmacology, Long Island College of Medicine*

BROOKLYN, NEW YORK

OPINION is divided as to the exact mechanism responsible for the elevated venous pressure of congestive heart failure. It is generally agreed, however, that two factors most commonly present in late congestive failure are a weakened heart and an increased blood volume. In animals and man there is strong evidence that simple acute increases in blood volume are not capable of producing prolonged elevations of venous pressure consistent with those found in heart failure (1-5). Likewise, experimental weakening of the myocardium has been found incapable of elevating venous pressure to significant levels (6, 7). Congestive failure with elevated venous pressure has been reported when both myocardial weakness and plethora have been induced in the same animal (8, 9). Yeomans *et al.*, however, have observed pulmonary edema in normal dogs during the height of massive rapid infusions (10).

Remarkably few experiments have been made in which both right and left auricular pressures have been measured simultaneously. Our aim in this investigation was to compare the changes in the right and left auricular pressure in acute heart failure produced by combining hypervolemia with myocardial weakness. The results in this report furnish further evidence that dissociation of the dynamics of the right and left sides of the heart can occur under appropriate conditions of hypervolemia, myocardial weakness and recovery.

## METHOD AND PROCEDURES

Data were collected from 13 cats, 8 female and 5 male, weighing 2 to 3.5 kg. Anesthesia was induced with Dialurethane solution intraperitoneally in doses of 0.6 to 0.7 cc/kg. body weight. Right and left auricular pressures were recorded with the chest opened and with artificial respiration through a tracheal cannula. Freshly paraffined glass cannulae were tied into the right and left auricles and pressures recorded with saline manometers to which heparin was added. The pressures were transcribed from the manometers to a smoked drum by Brodie Bellows which were calibrated before each experiment. Arterial pressure was recorded from the right carotid artery with a mercury manometer. Fluids and drugs were infused into the femoral vein. Electrodes attached to the right auricle were connected to a Thyatron stimulator having a frequency of 600 impulses/min. which generated current adequate to induce auricular fibrillation.

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Received for publication, September 22, 1948.

<sup>1</sup> Research Fellow in Physiology.

## RESULTS

*Normal Right and Left Auricular Pressures.* In 13 open-chest cats right auricular pressure ranged between  $-10$  mm. water and  $-43$  mm. with a mean value of 17 mm. In the same animals left auricular pressure ranged between 12 and 70 mm. with a mean value of 42. Values for left auricular pressure are comparable to those found by others in dogs with open chests (13), and to measurements of pulmonary venous pressure in unanesthetized closed-chest dogs (14). A similar range of right auricular pressure has been reported in normal mammals with the chest intact (dogs 5, 9, 11), (humans 11, 12). In only one animal was left auricular pressure lower than right. The mean ratio of right to left auricular pressure was 1 to 2.5 which approximates results found by Dexter using a saline manometer in intact

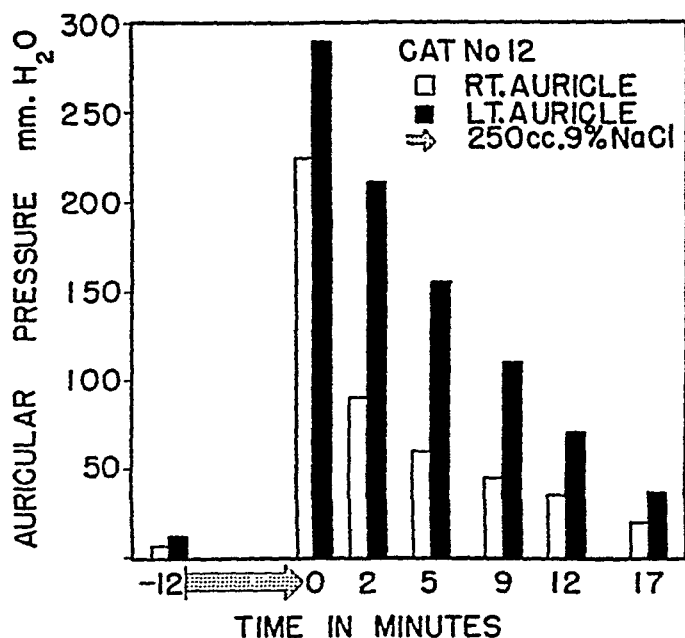


Fig. 1. PRESSURE IN RIGHT AND LEFT AURICLE before and after acute hypervolemia. Control auricular pressures represented at  $-12$  min. Zero time indicates level of auricular pressures immediately after estimated blood volume increased one-fold with physiological saline. Decline of auricular pressure to almost control levels plotted in succeeding 17 min.

dogs (15). The values obtained for resting auricular pressure in our cats, then, are in essential agreement with results of other workers.

*Right and Left Auricular Pressure of Acute Hypervolemia.* Landis and co-workers (5) and Yeomans *et al.* (10) have recently re-emphasized that simple massive increases in blood volume produced marked but only transient elevations of central and peripheral venous pressure with venous pressure dropping promptly after the infusion was discontinued. In one experiment in which left auricular pressure was measured it was found to rise more rapidly and to a much higher level than peripheral venous pressure (10).

Figure 1 illustrates a representative response to one-fold increases in estimated blood volume by rapid infusion of isotonic saline in 6 cats; 250 cc. of isotonic saline was given in 12 minutes. Right auricular pressure rose from 7 to 225 mm. water and left, from 12 to 290 mm.;  $5\frac{1}{2}$  minutes after the infusion was terminated right auricular pressure had dropped to 60 mm. and left, to 155 mm. Seventeen minutes after the end of the infusion auricular pressures were almost at control levels. Arte-

rial pressure dropped moderately at the onset of the infusion (115-100 mm. Hg) and had returned to control levels 17 minutes after end of infusion. This early drop has been noted by others (10) while Warren *et al.* (12) report that in normal human beings arterial pressure shows slight and random variations in response to infusion. Thus, as has been previously reported, acutely induced plethora produces marked but only transient elevations of right and left auricular pressure.

*Bilateral Elevations of Auricular Pressure with Quinidine.* It has already been demonstrated that large intravenous doses of quinidine produce severe myocardial impairment (16-19). This is characterized by a sudden drop in blood pressure and a slowing of pulse rate. Although the negative inotropic action of atabrine has been utilized in attempting to produce heart failure experimentally (20), to our knowledge quinidine has not been employed in this manner. In our experiments, quini-

TABLE 1. BILATERAL RISES IN AURICULAR PRESSURE (MM. OF WATER)

CAT	RIGHT AURICLE			LEFT AURICLE		
	Control	Quinidine	Change	Control	Quinidine	Change
<i>A. With Near Lethal Doses of Quinidine</i>						
3	19	51	32	78	100	22
5	25	49	24	20	38	18
7	30	60	30	69	110	41
9	32	88	56	81	103	22
Mean	26	62	36	62	88	26
CAT	CONTROL	AUR. FIB.	CHANGE	CONTROL	AUR. FIB.	CHANGE
<i>B. With Auricular Fibrillation</i>						
8	20	60	40	39	110	71
9	40	62	22	108	152	44
12	28	40	12	119	130	11
Mean	29	54	25	89	131	42

dine given intravenously in near lethal doses of 15 to 30 mg/kg. of body weight produced a severe arterial pressure drop of 30 to 80 mm. Hg, a bradycardia, a pulse pressure increase, cardiac dilatation and an elevation of auricular pressure, usually bilateral. Table 1 A shows the bilateral rises in auricular pressure of 4 hypervolemic cats given 25 mg/kg. of quinidine. Right auricular pressure rose an average of 36 mm. of water and left auricular pressure, 26 mm. Similar results were obtained in cats with normal blood volumes. Spontaneous recovery from the cardiotoxic action of quinidine was attended by a gradual return of arterial pressure, heart rate and cardiac size to control levels and by a bilateral drop of auricular pressure. Although complete recovery takes 2 to 3 hours (16), auricular pressures returned to prequinidine levels in 3 to 15 minutes. It is therefore apparent that severe weakening of the myocardium in normal and plethoric cats produces only moderate elevations of auricular pressure.

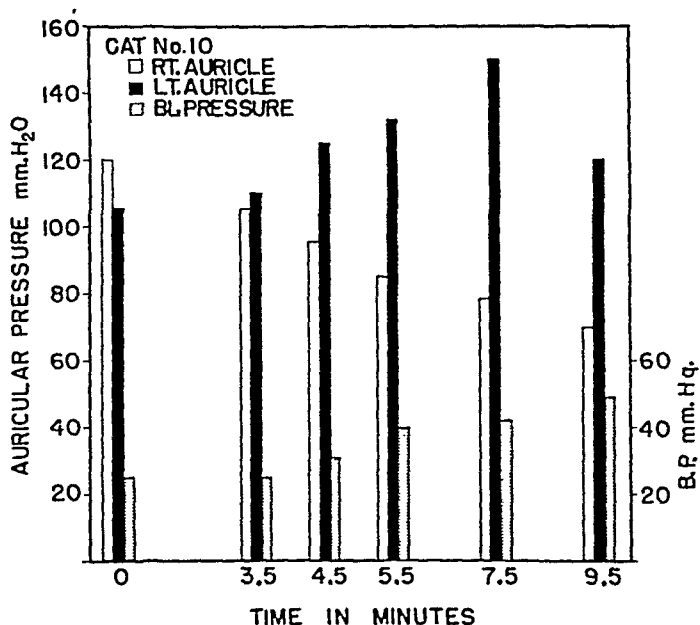
*Unilateral Elevations of Auricular Pressure with Quinidine.* In the same cat mani-

festing bilateral rises in auricular pressure after further infusion, a repetition of the near lethal dose of quinidine produced a unilateral elevation of auricular pressure. This consisted of an elevation of right auricular pressure and a drop in left. Table

TABLE 2. UNILATERAL RISES IN AURICULAR PRESSURE (MM. OF WATER)  
*Same cats as in table 1 with greater blood volumes*

CAT	RIGHT AURICLE			LEFT AURICLE		
	Control	Quinidine	Change	Control	Quinidine	Change
<i>A. With Near Lethal Doses of Quinidine</i>						
3	55	90	35	112	89	-23
5	20	78	58	95	70	-25
7	110	240	130	260	195	-65
9	90	115	25	150	130	-20
Mean	69	131	62	154	121	-33
CAT	CONTROL	AUR. FIB.	CHANGE	CONTROL	AUR. FIB.	CHANGE
<i>B. With Auricular Fibrillation</i>						
8	62	80	18	155	124	-31
9	63	90	27	190	115	-75
12	50	62	12	164	140	-24
Mean	58	77	19	169	126	-43

Fig. 2. SPONTANEOUS RECOVERY of heart weakened by quinidine. At zero time maximal depression of the heart with quinidine is represented by elevated auricular pressures and a low arterial pressure. Note that the first  $7\frac{1}{2}$  min. of early recovery are attended by a rising left auricular pressure as right auricular pressure declines and arterial pressure rises. Finally left auricular pressure begins to fall as arterial pressure continues to rise.



2 A lists the values obtained during these unilateral rises. Average rise in the right auricle was 62 mm. of water, while average drop in the left auricle was 33 mm.

The opposite type of unilateral auricular pressure elevation may also occur but only during the phase of early recovery from quinidine. Cat 10 (fig. 2) is a representative experiment of this type, showing an elevation of left auricular pressure and a decline in right auricular pressure in the recovery period after a near lethal

dose of quinidine. As recovery continued both auricular pressures gradually dropped to control levels.

The unilateral right auricular pressure rise during quinidine administration was attended by a more marked dilation of the right ventricle indicating predominantly rightsided weakness. Similarly in the unilateral left auricular pressure rise occurring during early recovery from quinidine the left ventricle was observed to be relatively more dilated than the right, proof that the right heart was recovering before the left.

*Effect of Auricular Fibrillation on Auricular Pressures.* Auricular fibrillation is also known to weaken ventricular contraction (5). The bilateral and unilateral right auricular pressure responses obtained during quinidine-induced myocardial

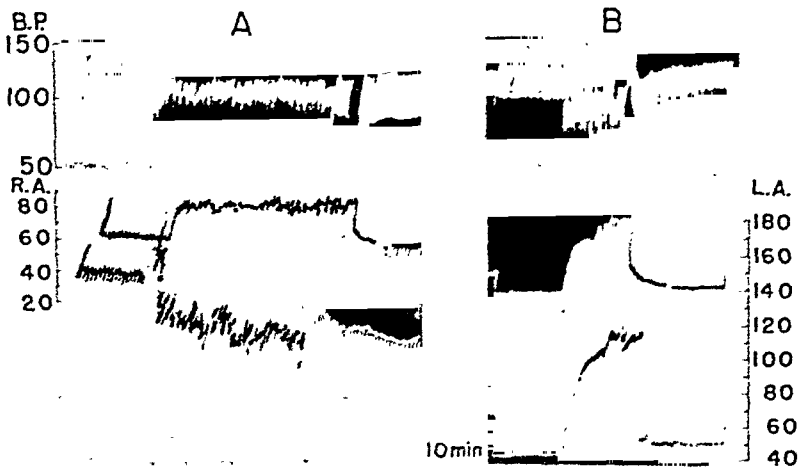


Fig. 3. UNILATERAL AND BILATERAL AURICULAR PRESSURE RISE of myocardial weakness. Reading from top to bottom: blood pressure (B.P.) in mm. Hg; time in seconds; right auricular pressure (R.A.) and left auricular pressure (L.A.) in mm. of water. The irregularity in the arterial pressure tracing indicates the period of electrically induced auricular fibrillation. A: Unilateral elevation of right auricular pressure with a drop in left auricular pressure in a hypervolemic cat immediately after blood volume increased by infusion. B: Bilateral elevation of auricular pressures in the same cat 10 min. later. Note that resting auricular pressures are significantly lower in B as compared to A.

weakness were duplicated with auricular fibrillation (fig. 3). Thus two methods of inducing cardiac weakness produced qualitatively similar results. Auricular pressures for the bilateral rise with auricular fibrillation are given in table 1 B, while pressures during the unilateral rise in the same three animals are tabulated in table 2 B. It is to be noted that in the unilateral response elicited with both quinidine and auricular fibrillation, the resting auricular pressures were at a higher level than those of the bilateral response.

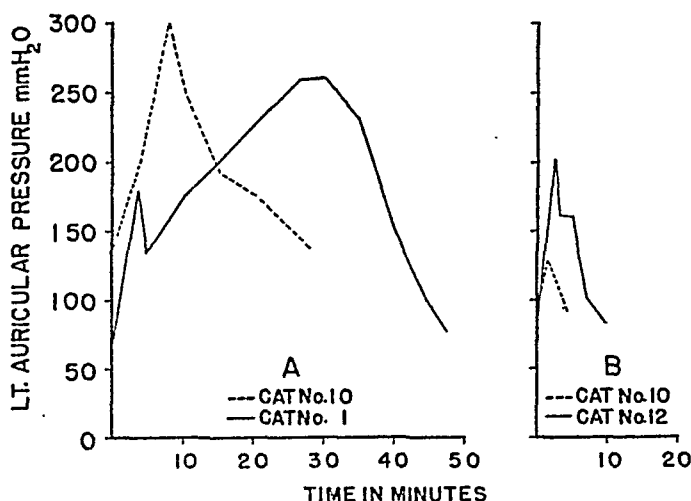
*Effect of Epinephrine and Pituitrin on Auricular Pressure.* It has been noted above that recovery of the heart weakened by quinidine may be attended by a rising left auricular pressure. In an attempt to elucidate this mechanism epinephrine, because of its positive inotropic action, was administered to the weakened heart and its effect on left auricular pressure noted.

Epinephrine in an intravenous dose of 20 to 40 gamma, administered to both nor-

mal and hypervolemic cats, caused a moderate and transient elevation of left auricular pressure, 30 to 71 mm. of water, lasting 1 to 9 minutes. There was no significant change in right auricular pressure. This was in sharp contrast to the marked, prolonged elevation of left auricular pressure produced by the same amounts of epinephrine in the hypervolemic cat whose heart had been weakened by quinidine. This effect is illustrated in figure 4 A, in which left auricular pressure rose 177 mm. and 165 mm. of water in *cats 1* and *10* respectively. Return to control levels took 28 and 47 minutes. The rise of right auricular pressure in *cat 1* was only 30 mm. water. In *cat 10* right auricular pressure dropped 44 mm. as left rose to 165.

In order to determine whether or not this left auricular pressure rise occurring in the weakened heart was a result of peripheral vasoconstriction or the direct stimulant action of epinephrine on the heart, posterior pituitary extract was used. With the use of posterior pituitary principles rises in left auricular pressure can only be attributed to peripheral vasoconstriction since cardiac performance is not enhanced

Fig. 4. EFFECT OF EPINEPHRINE AND PITUITRIN on left auricular pressure of the weakened heart. A: At zero time 20 and 40 gamma of epinephrine intravenously to *cats 1* and *10*, respectively. B: At zero time 2  $\mu$  of pituitrin intravenously to *cats 10* and *12* respectively. Note elevation of left auricular pressure is neither of comparable degree nor duration to that produced by epinephrine.



but on the contrary further depressed because of coronary vasoconstriction (7). Contrasted to epinephrine, pituitrin administered to the weakened heart had far less effect on left auricular pressure. In figure 4 B it is to be noted that two  $\mu$  of posterior pituitary extract produced only moderate and transient increases in left auricular pressure: 38 mm. in *cat 10* and 128 mm. in *cat 12*. Return to control levels occurred in 4 and 10 minutes. Right auricular pressure dropped very slightly in *cat 10*, i.e. 8 mm., and was unchanged in *cat 12*. Thus it is clear that the magnitude and duration of left auricular pressure rise is much greater with 20 to 40 gamma of epinephrine than it is with two  $\mu$  of pituitrin.

The positive inotropic action of epinephrine on left auricular pressure could only be ascertained by demonstrating that the degree of vasoconstriction produced with epinephrine and pituitrin in the amounts used was equal. Utilizing changes in mean arterial blood pressure as an index of the degree of vasoconstriction, it was observed that pituitrin produced at least an equal if not greater peripheral vasoconstriction than epinephrine. Thus 20 gamma of epinephrine in *cat 1* produced no elevation of arterial pressure as left auricular pressure rose 177 mm. of water.



In *cat 10*, 40 gamma of epinephrine elevated mean arterial blood pressure from 28 to 40 mm. Hg. However, arterial pressure had returned to control levels when the peak left auricular pressure rise occurred 7 minutes later. Pituitrin in a dose of 2 IU in the same cat caused a 10-mm. rise of blood pressure (40–50 mm. Hg) which persisted throughout the left auricular pressure rise and even after it had returned to control levels. In *cat 12*, 2 IU pituitrin elevated blood pressure 55 mm. Hg (25–80 mm.) at the time of peak left auricular pressure rise. Blood pressure continued to rise (90 mm.) five minutes after pituitrin was given and while left auricular pressure was dropping. When left auricular pressure had returned to control levels, the arterial pressure was still 50 mm. Hg above its pre-pituitrin value. These results indicate that although the peripheral vasoconstriction produced by epinephrine contributes in part to the unilateral left auricular pressure rise, its direct stimulant action on the weakened heart is the primary factor.

#### DISCUSSION

The inequality of myocardial weakness and recovery of the right and left hearts that appears manifest in the unilateral elevations of right and left auricular pressure lends itself to interpretation in terms of the volume-tension curve of cardiac muscle as promulgated by Frank (21), Kozowa (22), Starling (23), and others, (24, 25). In the previous paper it has been pointed out the heart may be considered to be a hollow elastic sphere. Increased venous return causes a distension of the cardiac chambers because of the higher distending pressure. At this increased heart muscle fiber length the mechanical advantage is decreased (27, 28). As a result of this the high venous distending pressure is not only maintained at a high level but may be actually increased because of the inability of the heart chamber to empty itself completely. Thus the heart is working at a high level of the volume-tension curve the events of which are summarized as follows: Changes in initial length change the mechanical advantage at which the heart-muscle fiber performs (28). The corollary of such an interpretation is that a high venous pressure is not the cause of distention of the heart chambers but rather that a high venous pressure results from such distention, i.e. an inability of the heart to empty completely. When a heart works on a higher level of this curve four events have occurred: 1) cardiac radius is increased; 2) there is decreased shortening of the myocardium during systole; 3) there is increased residual blood in the heart; and 4) auricular pressure is elevated.

In the experimental animal the heart can be made to ascend the curve by increasing venous return so that cardiac radius is increased. This can be accomplished by infusion or by decreasing cardiac output with quinidine or auricular fibrillation, which in effect increases venous return. Thus, during the bilateral elevation of auricular pressure with quinidine or auricular fibrillation the reduction in cardiac output on both sides of the heart amounts to an increased venous return. This causes both sides of the heart to work on a higher level of the curve and hence the elevated auricular pressures. The reverse situation prevails during the bilateral decline of auricular pressure accompanying recovery of the heart from quinidine.

Under certain conditions, particularly when the blood volume is very large and

the heart very weak, it has been shown that unilateral elevations of auricular pressure may occur. Assuming that the curtailment of cardiac output is equal on both sides of the heart, unilateral elevations of right auricular pressure with quinidine and fibrillation appear to indicate that the diastolic size of the right heart becomes disproportionately greater than the left. This was observed visually in our experiments; i.e., whenever right auricular pressure was greater than the left, the right ventricle was markedly dilated compared to the left. During such a state of affairs the right heart is working on a higher level of the volume elasticity curve and the left heart on a lower level. Under these conditions the right ventricle has a smaller coefficient of elasticity than the left ventricle but may maintain an output equal to the left ventricle because it is at a more advantageous volume/surface area position.

This interpretation is substantiated by the fact that the heart was always on a higher level of the volume elasticity curve prior to the unilateral auricular pressure response of myocardial weakness, contrasted to the bilateral auricular pressure response in the same animal (see mean control, auricular pressure, tables 1 and 2).

The unilateral elevation of left auricular pressure during the recovery from quinidine implies that the right heart is recovering more rapidly than the left, e.g. that the right heart is descending the volume-elasticity curve more rapidly than the left or that its cardiac radius is more rapidly approaching normal limits than that of the left heart. That the right heart may recover before the left has been suggested by Richards *et al.* (26). They observed that the right auricular pressure dropped as the vital capacity continued to decrease in a patient with heart failure following an infusion.

Patterson and Starling suggested that under certain conditions one side of the heart could fail before the other (27). They interpreted differences in the degree of rise of right and left auricular pressure in the heart-lung preparation as indicating such a situation. In this investigation it has been shown that auricular pressures can change in opposite directions. This would appear to furnish evidence that the dynamics of the right and left heart do dissociate. Finally, none of our observations support the contention of Henderson and Prince that the rise of right auricular pressure attending pulmonary engorgement is caused by a decreased capacity of the right ventricle as a result of displacement of the intraventricular septum during diastole (29). In our series of experiments dilation of the right auricle and ventricle always accompanied a unilateral rise of right auricular pressure.

#### SUMMARY

Acute heart failure was produced in open-chest cats by massive infusion with saline followed by sub-lethal doses of quinidine. This was usually attended by elevation of both right and left auricular pressures. In the same cat, further infusion and a second dose of quinidine was attended by a rise of right auricular pressure and a drop in the left auricular pressure. These results were duplicated with auricular fibrillation following massive infusion, thus showing that it is not a specific drug effect but that it is weakness of the myocardium that produces the changes in auricular pressure. As the animal recovered from the quinidine usually both right and left auricular pressure slowly declined to normal values. In the cats whose

blood volume had been greatly increased the left auricular pressure rose and the right auricular pressure fell in the quinidine recovery phase.

Epinephrine was used to accentuate the recovery phase in the unilateral left auricular pressure rise attending recovery from quinidine. Proof was advanced that it was largely the positive inotropic action of epinephrine on the heart rather than the system vasoconstriction which caused the left auricular pressure rise. This was accomplished by giving an equivalent dose of pituitrin which has purely vasoconstrictive properties.

The results permit the conclusion that in the presence of marked hypervolemia the onset of severe myocardial weakness and the recovery from that weakness may produce dissociation of the dynamics of the right and left ventricle. This phenomenon is simply explained by the observation that in this dissociation one ventricle is markedly dilated as compared to the other. Hence the deduction that under appropriate conditions one ventricle may function at a different level of Starling's Curve than the other.

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# HEAT LABILITY OF A DEPRESSOR SUBSTANCE PRESENT IN HUMAN URINE: EFFECTS OF SECTION OF VAGUS NERVES, LIGATION OF CAROTID ARTERY AND OF AUTONOMIC BLOCKADE UPON DEPRESSOR RESPONSE<sup>1</sup>

J. MAXWELL LITTLE, HAROLD D. GREEN AND JAMES I. BUMGARNER

*From the Department of Physiology and Pharmacology, The Bowman Gray School of Medicine of Wake Forest College*

WINSTON-SALEM, NORTH CAROLINA

IT HAS been reported previously that a decrease in the mean arterial pressure, a decrease in the peripheral resistance and an increase in the cardiac output follow the intravenous injection of urine into dogs (1). A method of assay for the depressor effect of urine is presented here, together with our analysis of the effects of heating the urine, the effects of vagus nerve section and of autonomic blockade upon the depressor response.

## METHODS

Mongrel dogs weighing 5 to 10.5 kg. were anesthetized with either pentobarbital sodium, 30 mg/kg., or amytal sodium, 50 mg/kg. One femoral artery was cannulated for recording mean arterial pressure using a mercury manometer, and one femoral vein was cannulated for making the injections. Unless otherwise indicated, all tests were performed on such animals. In a few dogs, the vagi were sectioned and in others the carotid arteries were compressed.

The various solutions were injected by syringe at a constant rate. Each injection, timed by an audible one per-second signal, took 20 seconds for completion. The injections were made at intervals of not less than 10 minutes, and before a subsequent injection was made the mean arterial pressure (MAP) had returned to the control value and had remained there for several minutes.

All urine samples were collected from healthy young men. The samples were filtered through filter paper if necessary. Immediately after collection they were placed in cellophane bags and dialyzed against running tap water for 24 hours. The bags were then transferred to distilled water which was usually changed four to six times during another 24-hour period. The dialysis was carried out in a refrigerator at an average temperature of 9° C. and all samples were frozen and stored in the low-temperature refrigerator until used.

Just prior to using, some of the dialyzed urine samples were heated in a constant temperature water bath at 40°, 60° and 80° C. for 120 minutes; some samples were heated in a boiling water bath for various time periods. All urine samples were adjusted to the original volume after heating by the addition of distilled water.

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Received for publication October 14, 1948.

<sup>1</sup> Supported by a grant from the Life Insurance Medical Research Fund.

Some dialyzed urine samples were concentrated by vacuum distillation at a temperature of 38° to 41° C. The distillate was saved for injection, and the residue was injected as such, or it was diluted to the original volume by the addition of distilled water. Histamine hydrochloride, 5  $\mu$ g/cc., was injected in doses of 2 to 6 cc. in order to compare the depressor effect of urine with that of histamine.

### RESULTS

*Typical Response.* Typical mean arterial pressure (MAP) responses to urine and histamine are reproduced in figure 1. They are from the same animal and were chosen to illustrate responses which we considered to be equivalent. In this instance 5 cc. of urine was equivalent to 10  $\mu$ g. of histamine.

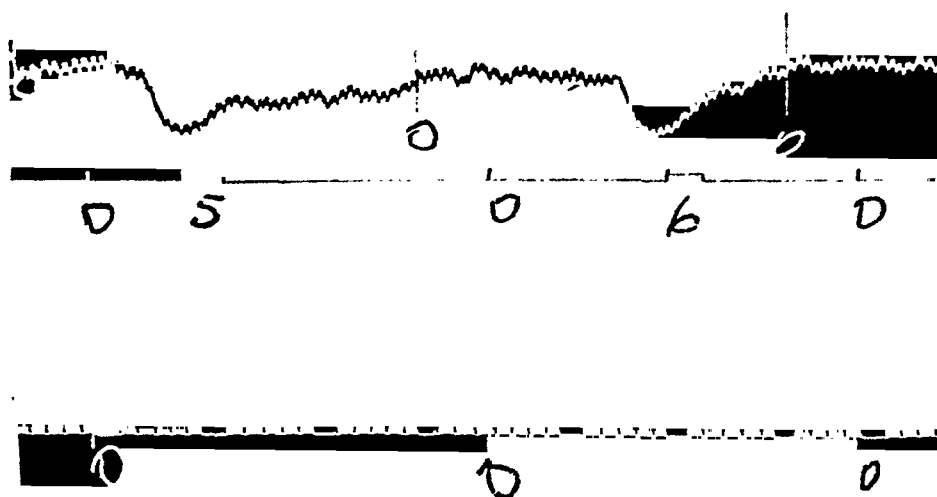


Fig. 1. COMPARISON OF DECLINE in mean arterial pressure in response to 5 cc. dialyzed urine (5) and to 10  $\mu$ g. histamine (6) injected intravenously into an 8-kg. dog anesthetized with sodium amytal (exp. N-31).

*Method for Expression of Response.* Possible methods for expressing responses of this type include *a*) maximum change in mean arterial pressure ( $-\Delta$ MAP), *b*) duration of response (sec.), *c*) response at some fixed interval after the injection and *d*) the integrated response (MAP·seconds), i.e., the area bounded by the base line and the MAP curve which results from the injection. For these studies we have used the maximum change in MAP. This may be expressed as absolute (mm) decline in mean arterial pressure ( $-\Delta$ MAP) or as percentage decline ( $\%-\Delta$ MAP =  $\frac{\text{control MAP} - \text{experimental MAP}}{\text{control MAP}} \times 100$ ). In figure 2 and table 1 we have analysed the response in 83 sets of injections using both methods of expression. Each set represented 2 or more injections of the same amount of substance (urine or histamine) into the same dog.

*Reproducibility of Response.* The maximum variability of response within each set of injections of urine and histamine in both intact and vagotomized dogs with respect to the mean response will be found in figure 2. It will be seen that the maximum variability increases with increasing mean response. However, most of the values for maximum variability lie below the line representing  $\pm 60$  per cent of the mean response. Greater responses and greater variability were found in the vagotomized animals. However, with the exception of histamine in the vagoto-

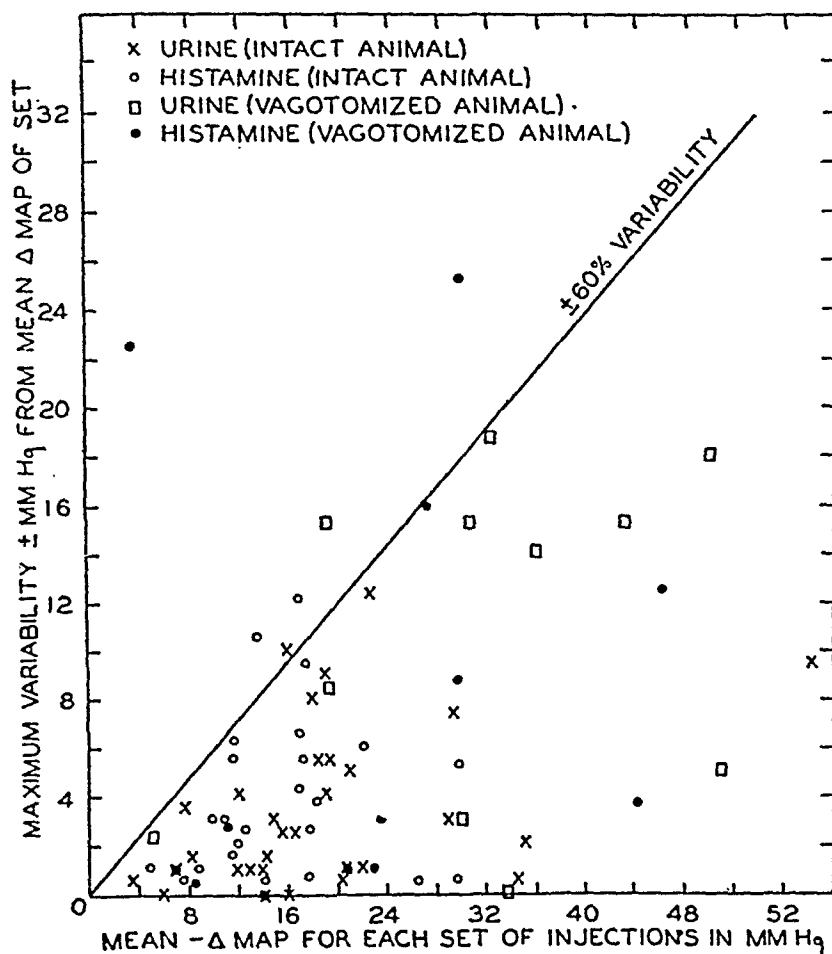


Fig. 2. RELATION between maximum variability within each set of injections and mean response within each set of injections. Each point represents the greatest single deviation from the mean response within each set.

mized animals, there was no difference in the percentage maximum variability of the substances injected in the two animal preparations.

An analysis of the reproducibility of the decrease in mean arterial pressure ( $-\Delta$ MAP;  $\%$  $-\Delta$ MAP) with repeated injections of the same dose of the same sample in the same dog is illustrated by the data in table 1. In almost 100 per cent of the sets of data in the intact dog the maximum deviation of any one response within a set did not differ from the mean  $-\Delta$ MAP of the set by more than  $\pm 12$  mm (table 1, I), and 68 to 72 per cent were within  $\pm 4$  mm (table 1, columns A and C). In the dogs with vagi sectioned (columns B and D) the variability was

greater. When the maximum variability of an individual determination in the intact dog was expressed as percentage of the mean response of the set approximately 60 per cent of the sets showed a maximum variation of less than  $\pm 20$  per

TABLE 1. VARIABILITY OF DATA  
Percentage of total sets in each group

	HISTAMINE				URINE			
	Intact Dog		Vagi Sectioned		Intact Dog		Vagi Sectioned	
	$-\Delta \text{MAP}$	$-\Delta \text{MAP} \times \frac{100}{\text{MAP}}$	$-\Delta \text{MAP}$	$-\Delta \text{MAP} \times \frac{100}{\text{MAP}}$	$-\Delta \text{MAP}$	$-\Delta \text{MAP} \times \frac{100}{\text{MAP}}$	$-\Delta \text{MAP}$	$-\Delta \text{MAP} \times \frac{100}{\text{MAP}}$
Deviation of individual data about mean for set	<i>A</i>		<i>B</i>		<i>C</i>		<i>D</i>	
$\pm 0$ to $\pm 4$ mm. Hg	67.8%		54.5%		71.8%		25.0%	
$\pm 4.1$ to $\pm 8$ mm. Hg	21.4%		0 %		15.6%		8.3%	
$\pm 8.1$ to $\pm 12$ mm. Hg	10.7%		0.1%		9.4%		8.3%	
$\pm 12.1$ to $\pm 16$ mm. Hg	0 %		18.2%		3.1%		41.6%	
$\pm 16.1$ to $\pm 20$ mm. Hg	0 %		9.1%		0 %		16.7%	
$\pm 20.1$ to $\pm 24$ mm. Hg	0 %		0 %		0 %		0 %	
$\pm 24.1$ to $\pm 28$ mm. Hg	0 %		9.1%		0 %		0 %	
I	99.9%		100.0%		99.9%		98.9%	
Number of sets in group	28		11		32		12	
Average of maximum deviation in each set from mean $-\Delta \text{MAP}$ of each set	3.3 mm. Hg		8.8 mm. Hg		3.4 mm. Hg		10.9 mm. Hg	
mean of $-\Delta \text{MAP}$ of all sets in each group	23.0 mm. Hg		29.3 mm. Hg		18.9 mm. Hg		32.0 mm. Hg	
Deviation of individual data about mean for set	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>	<i>J</i>	<i>K</i>	<i>L</i>	<i>M</i>
$\pm 0$ to $\pm 20$ %	57.1%	60.7%	45.5%	54.6%	65.6%	56.3%	25.0%	16.7%
$\pm 20.1$ to $\pm 40$ %	28.6%	21.4%	36.1%	18.2%	18.8%	28.1%	33.3%	58.3%
II $\pm 40.1$ to $\pm 60$ %	3.6%	3.6%	9.1%	18.2%	15.6%	12.5%	25.0%	25.0%
$\pm 60.1$ to $\pm 80$ %	10.7%	10.7%	9.1%	0 %	0 %	3.1%	8.3%	0 %
$\pm 80.1$ to $\pm 100$ %	0 %	3.6%	0 %	9.1%	0 %	0 %	8.3%	0 %
Number of sets in group	100.0%	100.0%	99.8%	100.1%	100.0%	100.0%	99.9%	100.0%
	28	28	11	11	32	32	12	12

$-\Delta \text{MAP}$  = decline in mean arterial pressure expressed as mm. Hg.

$\frac{-\Delta \text{MAP}}{\text{MAP}} \times 100$  = decline in mean arterial pressure expressed as % of control mean arterial pressure.

*A-D* = percentage of total sets in which the maximum deviation of an individual determination differed from the mean of the set by the number of mm. Hg indicated in the left-hand column.

*E-M* = percentage of total sets in which maximum deviation of an individual determination differed from the mean of the set by the percentile amount indicated in the left-hand column.

cent of the mean  $-\Delta \text{MAP}$  (table 1, *II*) and, particularly with urine, 100 per cent of the sets showed a maximum variation less than  $\pm 60$  per cent of the mean  $-\Delta \text{MAP}$  for the set (table 1, columns *E* and *J* and fig. 2). Expressed in this way the variability was still slightly greater in the vagotomized than in the normal dogs (table 1, columns *F* and *K*). Since one standard deviation includes 68 per cent of the

observations we might, thus, without actual computation, consider the standard deviation to be between  $\pm 20$  and  $\pm 30$  per cent of the mean  $-\Delta\text{MAP}$ .

The mean arterial pressure varied from time to time in the experiments. Since the response to a given injection might be expected to be different at high as compared with low control mean arterial pressure we also calculated the percentage change in mean arterial pressure ( $\%-\Delta\text{MAP}$ ) for each injection. As shown in columns *F*, *H*, *K* and *M* in table 1, the variability was no less with this mode of expression than when the response was expressed simply as maximum decrease in MAP recorded in mm. Hg. Since no decrease in variability of response was obtained by vagus section or by expressing the results as  $\%-\Delta\text{MAP}$ , the results in the remainder of the paper are expressed as  $-\Delta\text{MAP}$  (in mm. Hg) obtained on intact anesthetized dogs.

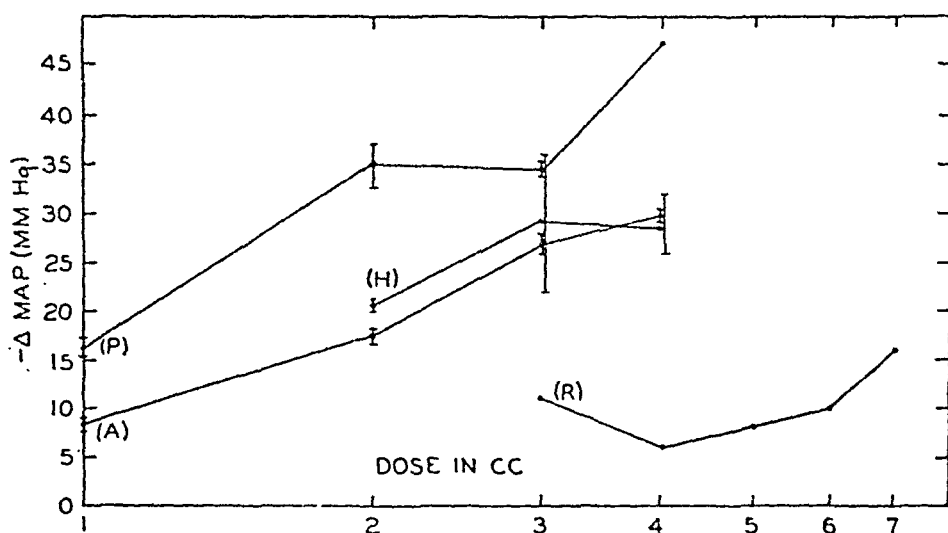


Fig. 3. COMPARISON of the log-dose and the average response with histamine and dialyzed urine in the same dog. *A*: histamine, 1 cc. = 5  $\mu$ g., 3 injections each dose; *P*: unheated urine, 2 injections each dose; *H*: urine heated in a boiling water bath for 30 min., 2 injections each dose; *R*: urine refluxed for 1 hr., 1 injection. Bar: maximum range. The same original urine sample was used in all injections.  $-\Delta\text{MAP}$  = decrease in mean arterial pressure in mm. Hg following injection. Anesthesia: amytal sodium (*Exp. N-12*).

From the above data it would appear that a response to a single injection would have to differ from the mean of a set of 2 or more injections by more than  $\pm 60$  per cent (2 standard deviations) to be significant and the mean  $-\Delta\text{MAP}$  of a set of 3 injections would have to differ from the mean of another set of 3 injections by  $\pm 50$  per cent to be significant ( $\sqrt{1/3 + 1/3} \times 60\% = 50\%$ ).

**Dose-response Relationship.** In 15 series of injections of histamine and urine into 12 dogs we explored the dose-response relationship. The response was expressed as  $-\Delta\text{MAP}$  in mm. Hg. The data were plotted on regular, on double log and single log paper. In no instance were consistently straight lines obtained. The most commonly obtained dose-response relationship was comparable to that in figure 3. According to these curves a 50 per cent increase in response would require doubling the dose and a 50 per cent decrease in response would be expected by halving the dose. On the basis of this data plus that in section 3 (*above*) it would appear that



the accuracy of this method of assay ( $\pm 2 \times$  standard deviation) would be  $\pm 100$  to  $-50$  per cent of the apparent concentration (or dose).

As shown in figure 3 there was usually a clear-cut shift in the locus of the plots upon heat treatment et cetera. Because of the variability of the plots we elected, insofar as possible, in making comparative assays to adjust the doses of the substances so that the same response was obtained with each. Comparison was then made on the basis of the required doses (fig. 4).

*Effect of Section of Vagus Nerves and of Autonomic Blockade upon Depressor Response to Urine.* The effects of bilateral vagal section, bilateral carotid artery ligation, the intravenous injection of 200 mg. of tetraethyl ammonium chloride (TEAC)<sup>2</sup>, and the intravenous injection of 10 mg. of Priscol (benzazoline hydrochloride, 2 benzyylimidazoline hydrochloride)<sup>3</sup> upon the depressor effect of urine will be found in

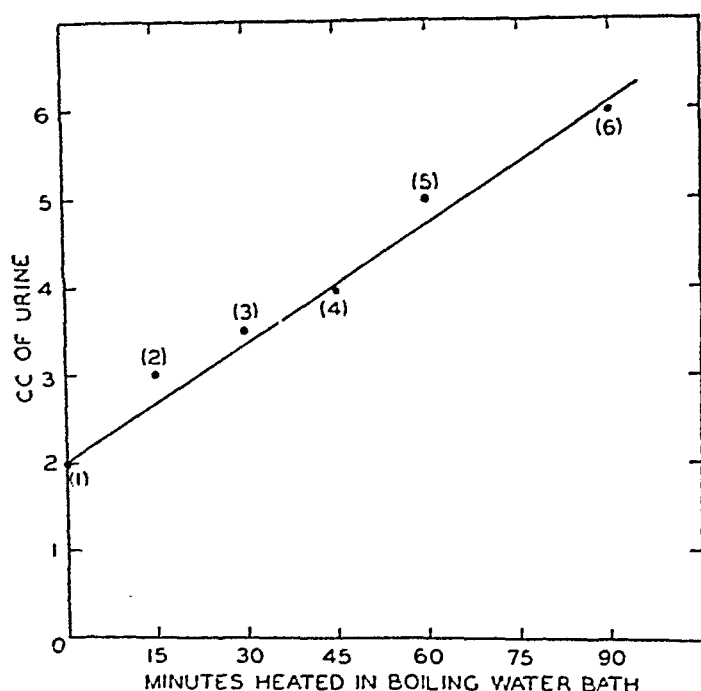


Fig. 4. VOLUME of dialyzed urine heated in a boiling water bath necessary to produce a decrease of approximately 13 mm. Hg in the mean arterial pressure of anesthetized dog. Anesthesia: amytal sodium. The same original urine sample was used throughout. Actual  $-\Delta$ MAP: (1) 13, (2) 16, (3) 14, (4) 13, (5) 17, (6) 13 mm. Hg.

table 2. It will be seen, in the last column, that vagal section increased the average urine depressor effect approximately 80 per cent, while subsequent bilateral carotid artery ligation with hypertension returned the urine depressor effect to approximately the control value. The injection of TEAC diminished the hypertension produced by carotid artery ligation and increased the depressor effect of the urine. The injection of Priscol increased the average mean arterial pressure, but, in contrast to carotid artery ligation, it caused a marked increase in the depressor effect of urine.

*Effects of Heating the Urine upon the Depressor Response.* a) *Effect of duration of period of heating.* The effects of heating the urine samples on the depressor response are presented in figures 3 and 4 and in table 3. Figure 3 shows the effects on the

<sup>2</sup> Supplied by Parke, Davis and Co., Detroit, Mich.

<sup>3</sup> Supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

depressor response produced by heating dialyzed urine in a boiling water bath for periods of 15, 30, 45, 60 and 90 minutes. The volume of urine necessary to give a  $-\Delta\text{MAP}$  of approximately 13 mm. Hg was determined for the control and for each of the heated urines. This volume was plotted against the time the urine was heated. It will be seen that a straight line function results.

TABLE 2. EFFECT OF VAGAL SECTION, CAROTID ARTERY LIGATION, INJECTION OF 200 MG. OF TETRAETHYLAMMONIUM CHLORIDE (TEAC), AND INJECTION OF 10 MG. OF PRISCOL ON THE HYPOTENSIVE RESPONSE TO THE INJECTION OF POOLED FRESHLY-VOIDED (NON-DIALYZED) URINE  
*Exp. N-2. Pentobarbital Sodium Anesthesia*

SUCCESSIVE PROCEDURES	NO. OF INJECTIONS	VOL. URINE INJECTED RANGE IN CC.	PRE-INJECTION MAP		$-\Delta\text{MAP}^1$ RANGE	$-\Delta\text{MAP}/\text{CC. URINE}$	
			Range	Average		Range	Average
			mm. Hg	mm. Hg		mm. Hg	mm. Hg
Control.....	9	4-6	154-168	161	10-18	1.7-4.5	2.8
Both vagi sectioned.....	4	6	159-172	164	27-32	4.5-5.3	5.0
Both carotids ligated.....	8	6-8	188-208	199	10-32	1.3-4.0	2.5
TEAC.....	4	4-6	100-179	130	17-44	2.8-7.3	4.6
Priscol.....	2	4-6	173-185	179	28-64	7.0-10.6	8.8

<sup>1</sup>  $-\Delta\text{MAP}$  = decrease in mean arterial pressure.

TABLE 3. EFFECT OF HEATING DIALYZED URINE ON THE HYPOTENSIVE RESPONSE TO ITS INJECTION  
*Exp. N-14. Amytal Sodium Anesthesia*

URINE NO.	HEATING		NO. OF INJECTIONS	VOL. INJECTED RANGE	$-\Delta\text{MAP}^1$ RANGE	CC./15 MM. HG $-\Delta\text{MAP}$	
	Temp.	Time				Range	Average
	°C.	min.		cc.	mm.Hg		
13A	0	0	9	1-4	7-28	1.4-2.5	1.9
13B	40	120	2	3-3.5	18-20	2.5-2.7	2.6
13C	60	120	4	3-3.5	16-22	2.1-2.8	2.5
13D	80	120	3	3-4	16-24	2.5-2.8	2.7
13E	B.W. <sup>2</sup>	30	8	2-6	8-19	2.1-6.3	3.8
13F	B.W.	60	3	6-6.5	10-26	3.8-10.0	6.6
13G	B.W.	90	3	9-10	14	7.2-9.4	7.9
13H	B.W.	120	3	12-14	10-24	5.9-18.0	13.5

<sup>1</sup>  $-\Delta\text{MAP}$  = fall in mean arterial pressure.    <sup>2</sup> B.W. = boiling water bath.

b) *Effect of boiling water bath as compared with refluxing.* While the relationship between log-dose and the response ( $-\Delta\text{MAP}$ ) in figure 1 is not strictly a straight-line function for either histamine or the urine samples, it is clear that when urine, heated in a boiling water bath for 30 minutes (*H*) or refluxed for 1 hour (*R*), is compared with unheated urine (*P*) there is a decrease in the depressor response. Put another way, an increased dose of (*H*) or (*R*) is required to produce the same response as that obtained with (*P*). It is to be noted that the greatest destruction of the depressor factor was produced by the reflux condensor treatment.

c) *Effect of temperature at which urine is heated.* The last column in table 3 shows that the depressor effect of urine is decreased from the control effect by heating for

120 minutes at 80° C. or below, but the temperature of heating within this range appears to be immaterial. However, the decrease in depressor effect of urine when it is heated in a boiling water bath is dependent upon the duration of heating.

d). *Effect of vacuum distillation.* Table 4 shows that when dialyzed urine is distilled *in vacuo* the depressor substance remains in the residue almost entirely. This is illustrated by comparing 17 P (unheated) with 17 RD (residue diluted to the original volume with distilled water). There appears to be little or no decrease in the depressor effect as a result of this procedure.

TABLE 4. EFFECT OF VACUUM DISTILLATION OF URINE ON THE HYPOTENSIVE RESPONSE TO ITS INJECTION  
*Amytal Sodium Anesthesia. Distillation Temperature, 38-41° C.*

EXP. NO.	URINE NO. <sup>1</sup>	NO. OF INJECTIONS	VOL. INJECTED RANGE	-ΔMAP <sup>2</sup> RANGE	CC./15 MM. HG-ΔMAP	
					Range	Average
N-18	17P	4	0.3-0.5	7-20	0.38-0.65	0.57
	17RD	1	0.5	13		0.58
	17D	3	5-20	5-10	15.0-30.0	20.6
N-24	17P	4	1-1.5	9-18	1.3-2.5	1.8
	17D	2	30	11-15	30.0-37.5	33.7
	17R <sup>3</sup>	5	0.2-1	10-38	0.22-0.40	0.31
	17RD	2	1.5	14-20	1.1-1.6	1.4
N-25	21P	2	2	11-21	1.4-2.7	2.1
	21RD <sup>4</sup>	2	2-3	10-13	3.0-3.5	3.3
	21D	5	7-10	15-29	5.0-8.8	6.0

<sup>1</sup> P = unheated dialyzed; D = distillate; R = residue from distillate; RD = residue diluted to original volume with distilled water. <sup>2</sup> -ΔMAP = fall in mean arterial pressure. <sup>3</sup> Concentrated 3.45 times. <sup>4</sup> Distilled to dryness, dissolved in 25 cc. distilled water: concentrated 20 times.

#### DISCUSSION

A depressor substance, callicrein or padutin, present in urine, pancreatic juice and intestinal secretions has been described by Frey and co-workers. This material is heat labile. Werle (3) has reported that callicrein obtained by pancreatic fistula in the dog is reduced in activity 85 to 95 per cent when the preparation is heated for 3 minutes over an open flame. Westerfeld *et al.* (4) reported that heating a callicrein preparation in a boiling water bath for 20 to 30 minutes markedly diminished but never completely abolished the hypotensive effect. Wollheim (5) reported that callicrein was destroyed in 10 minutes when heated at 100° C. in neutral, acid or alkaline media.

In table 3 it will be seen that heating dialyzed urine for 120 minutes at 40, 60 and 80° C. diminished the depressor effect of the urine approximately 30 per cent, and that the decrease in depressor effect was not related to the temperature at which the urine was heated. This change is within the apparent limits of error of the method. In contrast, heating the urine for 30 minutes in a boiling water bath decreased the depressor effect approximately 55 per cent. The decrease in depressor effect increased as the duration of heating at this temperature was prolonged, i.e.

to 13 per cent of the control after 2 hours. This change is significant. We interpret these data as evidence for two depressor substances present in urine. One of these is quite heat labile, being destroyed at temperatures below  $100^{\circ}\text{C}$ . and is probably callicrein, while the other substance, although still heat labile at  $100^{\circ}\text{C}$ ., is much more resistant to heating than is callicrein. This interpretation is strengthened by the data in figure 2 where refluxing the urine sample for one hour did not completely destroy the depressor activity, although it was less than the control activity or the activity of urine heated in boiling water for 30 minutes.

Wollheim (5) has previously reported an urinary depressor substance, which he called depressan. He found that this substance was active after heating at  $100^{\circ}\text{C}$ . for 10 minutes in a neutral solution, was active after 5 minutes heating in acid solution, but was destroyed after 30 seconds heating in alkaline solution. It is quite probable that the depressor substance reported here is very similar to if not identical with the depressor substance of Wollheim.

From the data given in table 2, it would appear that the site of action of the depressor substance reported here is primarily on the peripheral vessels. When a vasoconstriction was elicited by bilateral carotid artery ligation the depressor effect was diminished by 50 per cent from the previous value obtained after bilateral vagal section. However, when TEAC, which has been shown by Acheson and Pereira (6) to block autonomic ganglia, was injected the depressor effect of the urine returned to approximately the value found before carotid artery ligation. When Priscol, which is reported by Chess and Yonkman (7) to be sympatholytic and adrenolytic in reference to the sympathetic neural control of blood pressure, was injected it caused a marked increase in the depressor effect of the urine. This may be due to the more complete abolition of tonic vasoconstriction following Priscol, or to a reversal of effect of another substance with constrictor properties, i.e., an epinephrine-like substance.

The possible relationship between this depressor substance and the problem of hypertension is obvious. Hypertension may be the result not only of an excess quantity of angiotonin or hypertensin (as has been stressed by much recent work), but a decrease in the amount of the urinary depressor substance may also contribute to the elevation of arterial blood pressure. In essential hypertension it is possible that there is a decrease in the depressor substance, which might lead to an imbalance in the regulation of peripheral resistance. Wollheim (5) has reported that his depressan is either entirely missing or at least markedly decreased in the urine of patients with essential hypertension. On the other hand, patients who had a primary renal lesion and also had hypertension did not show any significant alteration in the amount of depressan excreted in the urine. The site of formation of the urinary depressor substance reported here is not known nor is it known whether or not the substance is present in blood.

#### SUMMARY

The depressor effect of urine was studied by recording the decrease in mean arterial pressure ( $-\Delta\text{MAP}$ ) produced by intravenous injection of the urine into anesthetized dogs. Analysis of results suggests that the mean of the response to a set

of three injections must differ by  $\pm 50$  per cent from that of another set to be significant, and that the apparent concentration of a dilator substance estimated by this method may differ from the true concentration by  $-50$  per cent to  $+100$  per cent.

Evidence is presented that, in addition to callicrein, there is present in normal dialyzed human urine a substance which causes a decrease in the mean arterial pressure of dogs anesthetized with amytal sodium when the urine sample is injected intravenously. This depressor substance is somewhat heat labile at  $100^{\circ}\text{C}$ ., but it is not completely destroyed by refluxing the urine sample for one hour. The urine sample may be concentrated by vacuum distillation at  $38^{\circ}$  to  $40^{\circ}\text{C}$ ., and the depressor substance is recovered almost quantitatively in the residue from the distillation.

The apparent activity of the depressor substance is increased by bilateral vagal section; it is decreased by subsequent bilateral carotid artery ligation. Intravenous injection of 200 mg. of tetraethyl ammonium chloride (Etamon) and injection of 10 mg. of benzazoline hydrochloride, 2 benzyylimidazoline hydrochloride (Priscol) increase the depressor response to the urine. The possible relationship of this substance to hypertension is discussed.

The authors are indebted to Miss Emily Angell and Mr. R. H. Holzworth for valuable assistance in these experiments.

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# EFFECTS OF COLD ON INFANT RATS: BODY TEMPERATURES, OXYGEN CONSUMPTION, ELECTROCARDIOGRAMS<sup>1</sup>

JANET FAIRFIELD

*From the Department of Physiology, University of Rochester, School of Medicine and Dentistry*

ROCHESTER, NEW YORK

**T**HERMOREGULATION in mature animals has been studied especially by physiologists who realized the important contribution of this regulation to the constant internal environment. The stable body temperature puts mammals in a different class from cold-blooded animals, as far as survival is concerned, for they are often able to carry on the necessary functions of finding food and protecting themselves against enemies, almost regardless of the environmental temperature.

The responses of adult animals to cold environments have been well established by Barbour (1), Giaja (2) and others. The development of these responses in immature animals, however, has not been as thoroughly studied. Hill (3) presented measurements of body temperatures, during cooling of rats at different ages, and found that the greatest gain in the resistance to lowering of the body temperature occurred between 18 and 30 days of age. Antoschkina (4) measured oxygen consumptions during experimental cooling of young rats. Her conclusions were that rats are poikilothermic at birth, not showing evidence of chemical regulation until one to two weeks of age, or physical regulation until three to four weeks of age.

The changes in the body temperature, the heat production and the electrocardiogram, during and after single exposures at different stages of development, have as yet not been thoroughly studied and are the subject of this paper. Thermoregulation is concerned with the gain of heat from the environment, with internal heat production, and with heat loss. In cold environments, heat gain from environment, of course, is not a factor. In this investigation, cooling was observed by body temperature determinations and heat production by oxygen consumption measurements. Since the circulatory system is the recognized distributor of body heat, electrocardiographic data were taken to study the condition of the heart in the maintenance of the circulation. Observation of the skin color was the only practical indicator of peripheral circulation.

## PROCEDURE

Forty-one white rats from 8 litters of the Wistar strain, of 0 to 17 days of age, were used. Each rat was exposed, in a sealed modified Fenn respirometer, to a control water bath of 35°C., so its

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Received for publication October 11, 1948.

<sup>1</sup> The author wishes to express appreciation to Dr. Edward F. Adolph for help and criticism throughout this work and to the Aeromedical Laboratory, U. S. Air Forces, for financial support of the project.

normal body temperature, electrocardiogram, metabolism and general neuromuscular behavior could be observed. Then the respirometer was removed to the experimental cold water bath of 20°, 10°, 5° or 2° for two hours, usually, in which period the animal's reactions to the exposure were studied; finally the respirometer containing the animal was transferred back to the control 35° bath for rewarming and kept there until the animal was at its pre-exposure temperature. The 35° point was chosen for the control bath because this, coupled with the animal's metabolism, brought the body temperature up to about 37°.

The respirometer was a differential volumeter which permitted an accurate reading of the animal's oxygen consumption at all times except during the severest changes in the temperature of the chamber. The rat lay in the glass vessel of 157 ml. volume on a metal screen above soda lime in an atmosphere of oxygen. Records of the oxygen consumption were kept continuously, except for the first few minutes of each immersion and the data presented in this paper are all corrected by a small respirometer factor and to 0°C. and 760 mm. pressure.

The intraperitoneal temperature was recorded every six minutes by a Brown recording potentiometer, fine-gauge thermocouples being threaded through the abdominal wall from one side to the other. This was found experimentally to be a more accurate measure of the core temperature of the body than was the rectal temperature, since it changed more slowly than the rectal temperature during cooling and warming. Some experiments included records of rectal and surface temperatures, as well as intraperitoneal and air temperatures inside the respirometer.

Electrocardiograms were taken at intervals by means of subdermal steel electrodes leading to a Sanborn electrocardiograph. Lead II was their approximate position.

## RESULTS

*Intraperitoneal Temperature.* The intraperitoneal temperature from birth up to 17.5 days of age depends almost entirely on the environmental temperature. When the animal is in the 35° control bath, the newborn's intraperitoneal temperature is not above that of the bath. However, at 3 or 4 days of age, it is about 1° over the bath temperature and this gradient increases slowly with age, the oldest animal showing a body temperature 3° over that of the bath after about 40 minutes.

This gradient effect may be due to several factors. The larger rat has proportionally less surface area over which to lose heat and more tissue to produce heat. Moreover, the 17-day rat has a coat of baby fur, as well as an appreciably thicker skin and body, to increase the insulation against heat loss.

During cooling, however, the intraperitoneal temperature is almost completely dependent on the environmental temperature. When the respirometer containing the animal is exposed to the cold bath, the body temperatures begin dropping immediately; the colder the bath, the more precipitous the fall. The rate of fall slows and almost plateaus as it approaches the air temperature of the respirometer. In general, with experimental temperatures down to 5°C., the difference which the animal can maintain between its temperature at the plateau and the external temperature is nearly as great as that which it could maintain at the control environmental level of 35°. These results agree in general with Gulick's (5) work on young rats. Figure 1 shows the body temperature of an 8.5-day rat before, during and after a 20° exposure.

A series of similar cooling experiments was done on 5 formalin-injected animals to compare the rate of temperature change of these non-metabolizing animals with the live ones. The gradients between the internal and the environmental temperatures for the dead rats during cooling are almost identical for all ages and weights, less than 1.5°C. They exhibit typical Newtonian cooling curves, which the living rats do not do. The live animals, on the other hand, show a progressive increase in

their ability to maintain a wider gradient for a longer time as they grow older. Since, as will be shown later, the metabolism falls during the cooling, the ability to maintain this gradient may be due, to a considerable degree, to an increasing ability to regulate peripheral blood flow.

However, there is no evidence of this ability to keep the body warmer than the environment during an exposure of  $2^{\circ}\text{C}$ ., in my apparatus, even in a rat of 11.5 days and 21 gm.; the gradients of the live and dead animals show the same curves with time. It appears therefore that, if the exposure is severe, the animal shows no ability

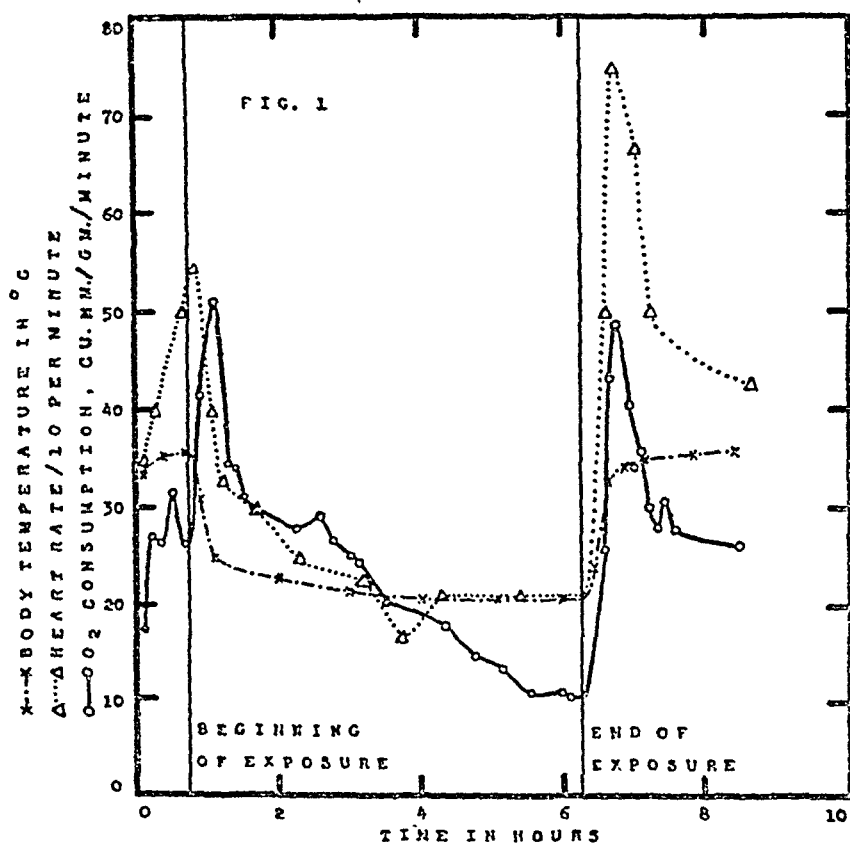


Fig. 1. A TYPICAL COOLING EXPERIMENT, done on an 8.5-day-old, 14.4-gm. rat at  $20^{\circ}\text{C}$ . The responses of the body temperature, heart rate and oxygen consumption are shown during the  $35^{\circ}$  control period, the  $20^{\circ}$  exposure and the final  $35^{\circ}$  recovery period.

to preserve its body temperature. This is probably due to the paralyzing action of cold on the nervous system, preventing the protective reflexes.

There is no correlation between the rate of fall of body temperature and death, nor between the depth of body temperature and death, except that the 4 animals which died, out of the 41 experimented on, died between  $3.5^{\circ}$  and  $7.5^{\circ}\text{C}$ . Most, however, survived temperatures as low as  $2.5^{\circ}$ . Apparently there is much variation in individual susceptibility to cold.

The rise of body temperature during the rewarming in the  $35^{\circ}$  bath was faster than the fall had been, judged by the time required for half completion, because heat production is working toward a rise in temperature during the rewarming and against the fall during cooling. Even animals which eventually died during the rewarming,



and which showed even a faulty electrocardiogram, had enough heat production to cause their rewarming temperature curve to be faster than the cooling curve had been.

*Oxygen Consumption.* From figures 2 and 3, and other data, the following comparisons can be made. The oxygen consumption/gm. of rat, measured after the animal had become equilibrated in the  $35^{\circ}$  bath for about an hour during the initial control period, was quite constant with age. It ranged from 21 to 36 cu. mm/gm. body/min. When the animals were transferred from the  $35^{\circ}$  control bath to the  $20^{\circ}$  bath, those over three days of age immediately increased their  $O_2$  consumption, some-

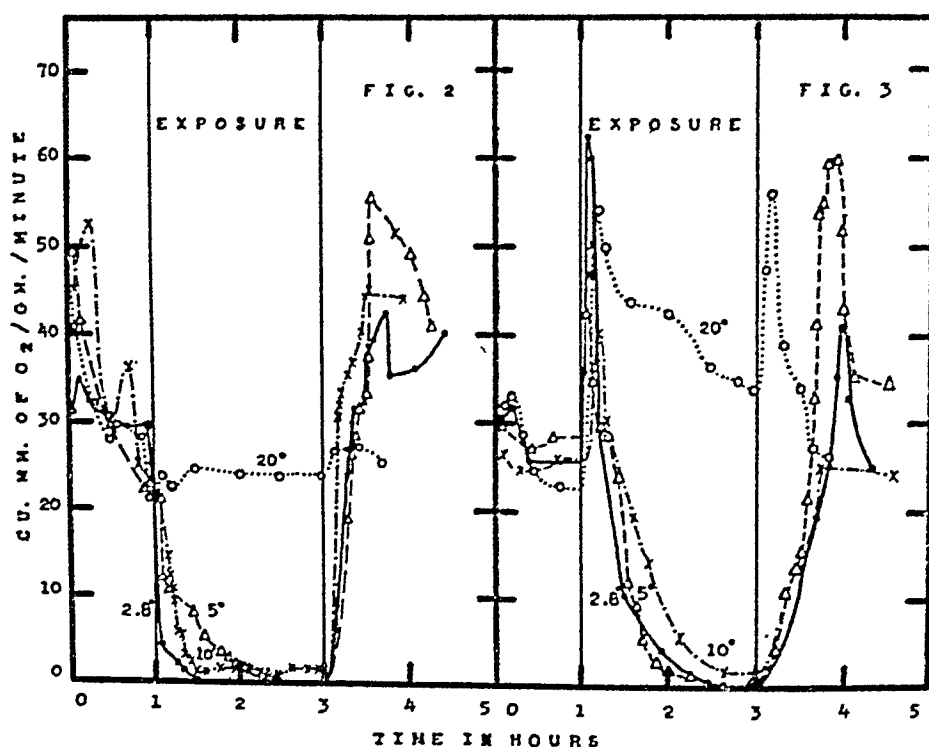


Fig. 2. OXYGEN CONSUMPTION before, during and after 4 different exposures of 2 to 3-day-old, 6 to 9-gm. rats.

Fig. 3. OXYGEN CONSUMPTION before, during and after 4 different exposures of 10 to 11-day-old, 18 to 20-gm. rats.

times to a large degree; the older animals increased theirs by a maximum of 170 per cent (in a 15-day-old animal), within 15 minutes of the beginning of the exposure. This peak metabolism was, however, not maintained for more than one-half hour and then gradually fell to the control  $35^{\circ}$  level over a one to four-hour period in the  $20^{\circ}$  bath. This bath proved to be the most effective one of those tested in stimulating metabolism.

Animals under about three days of age showed no increase in oxygen consumption at  $20^{\circ}$ , but just a gradual fall, and even the older animals showed a fall after their peak metabolisms—to a minimum of 35 per cent of the control level in exposures over five hours long.

On exposure to the  $10^{\circ}$  bath, only animals over eight days of age showed an increase in oxygen consumption and the highest metabolism recorded for them was 100

per cent over normal, in an 11-day rat. At the lower temperatures, the results were less consistent, but there was a definite decrease in the number of animals which manifested self-protection by increasing their metabolisms. The degree to which they were able to raise these metabolisms likewise fell markedly.

In explaining the apparent decrease in stimulation of metabolism with an increase in the severity of the exposure, probably a large factor is that the colder baths reduce the body temperature too quickly for the animal to mobilize its responses. It was pointed out above that the colder the bath, the faster the fall in body temperature. Cold is well-known as an anesthetic and narcotic and this depressant effect prevents any excitatory response from occurring. The mechanisms involved in increasing the metabolism are discussed later.

In all of the animals experimented on below 20°, immediately after the beginning of the cold exposure or if there was an increase in metabolism immediately after the peak metabolism, there was a precipitous drop in oxygen consumption which slowed as it approached zero. In the 20° exposure, however, animals older than nine days showed the stimulated metabolism only during the first two hours of the exposure, but even this was beginning to fall toward the normal 35° level. Apparently, between the 20° and 10° exposures, there is a break somewhere in the animal's ability to keep its metabolism high. Possibly this break would come at the lethal point for adult animals, between 13° and 16°C. It seems that there is some factor in the adult rat which causes death in this temperature range and it seems possible that something similar, but non-lethal, is breaking down in the young rats also.

It was constantly found that the metabolism fell to zero, as far as could be determined with this respirometer, in all animals up to 10 days of age on exposure to 3° or lower. The older animals were apparently able to maintain their metabolisms at about 0.5 cu. mm. O<sub>2</sub>/gm. rat/min. until the end of the exposure. These amazingly low or non-existing metabolic rates and the apparent cessation of visible respiratory movements by no means predicted death. This lack of breathing lasted up to 108 minutes, followed by complete recovery of the animal.

At the end of the exposure, between 20 and 25 minutes after the beginning of rewarming from 5° or lower temperatures and between 15 and 20 minutes after the beginning of rewarming from 20°, the oxygen consumption began to rise at about the same rate as it had dropped and was back at its pre-exposure level within 42 minutes of the beginning of rewarming, the slower ascents being in the colder rats.

The first breathing movements, however, are evident slightly sooner. After 5° or colder, the first gasping movements were seen at 15 to 25 minutes in the 35° bath; after 10°, at 5 or 6 minutes in the 35° bath.

Often the metabolism continued rising, to as much as 100 per cent (an av. of 60%) above the pre-exposure level, and slowly descended to normal within two hours of the beginning of rewarming. The largest increase over normal occurred in those animals exposed to the lowest temperatures, 5° or lower, or to the slightest exposure, 20°. It is difficult to find an explanation for this increase since there was no obvious correlation of appearance of this 'overshoot' with age, length of exposure, depth of oxygen consumption fall during cooling, increased metabolism at the beginning of cooling or with shivering movements, although muscle tension may have been in-

volved. Nor did there seem to be any correlation with the length of time or degree that the animal was anoxic during the cooling or rewarming, so the increase does not seem to be an oxygen debt.

It is difficult to state with certainty the method by which the metabolism is increased in the infant rats. As far as muscular activity is concerned, no increase in movements could be detected during the increased metabolism in the rewarming period and, although there was usually some struggling at the beginning of cooling when there was an increase in metabolism, the increase and the struggling sometimes appeared independently. No shivering movements were observed in the infant rats, even in the older ones. Muscular rigidity and tonus, however, may be a factor in the augmentation of oxygen consumption of these animals. Barbour, McKay, and Griffith (1), Dill and Forbes (6) and Swift (7) have all reported that muscular rigidity and tonus are capable, in man and rats, of producing an increased metabolic rate—up to 36 per cent, Swift (7) states—without actual shivering.

As far as hormonal stimulation is concerned, it is interesting to speculate as to the possibilities of increased activity of the adrenal and thyroid glands. It has been demonstrated by Tyslowitz and Astwood (8) and Giaja and Chahovitch (9) that the adrenal cortical hormones are necessary for resistance against cold. The difficulty in applying this fact to my experiments lies in the questions of whether the adrenals are actually functioning at birth in rats and whether they or the thyroid respond to stimulation in the short periods involved in these experiments.

*Electrocardiogram and Pulse Rate.* The electrocardiograms taken during both control and cooling periods show that the heart rate is lowered almost linearly with a decrease in body temperature (fig. 4). Crismon (10) and Hamilton, Dresbach and Hamilton (11) likewise found a linear relationship between body temperature and heart rate of adult rats. The line for the newborn rats, however, is located toward the lower body temperatures. This is perhaps to be expected since the newborn animal's heartbeats stop at a lower temperature than do the adult animals'. Adult rats' hearts stopped beating at between 16° and 12.5°C. rectal temperature (11), while the newborns' stopped between 9° and 3.5°C. Moreover, this asystole was lethal for the adult animals, while it was not for the newborns.

These results suggest that the newborn, incompletely developed rat resembles a cold-blooded animal in its ability to survive asystole during adverse external conditions with no apparent permanent damage. One rat which we studied lowered its normal heart rate of 860/min. to 12/min. during hibernation.

The rate of conduction of the cardiac impulse slows during cooling in proportion to the slowing heart rate. This is to be expected, since the cardiac cycle length is inversely proportional to the cardiac rate, and the P-R interval and QRS duration increase linearly with cycle length. Crismon (10) and Hamilton *et al.* (11) found this to be true also of adult rats and Clark (12) found it in rabbit and frog isolated hearts.

During the cooling experiment there is some increase in voltage of R-waves, as Crismon (10) reports for adult rats, immediately after the beginning of cooling. This is followed by an enormous decrease in voltage during the decrease in heart rate and conduction rate.

There is apparently no temporary increase in heart rate, as there is in oxygen consumption, immediately after starting the exposure to cold. This is illustrated by figure 4, in which the heart rate of a 9.5-day-old rat is plotted against its body temperature during the control, cooling and rewarming periods of a typical experiment. The heart rate shows a precipitous drop which slows as it approaches a steady rate which, in many cases, as in this animal, was zero.

Occasional records of R-waves at irregular intervals, as the animal becomes very cold, indicate auricular fibrillation although, in general, no irregularity of beat occurs before the cessation of beat. The lack of ventricular systole lasts until just after the beginning of warming, which has meant asystole for as long as 82 minutes with

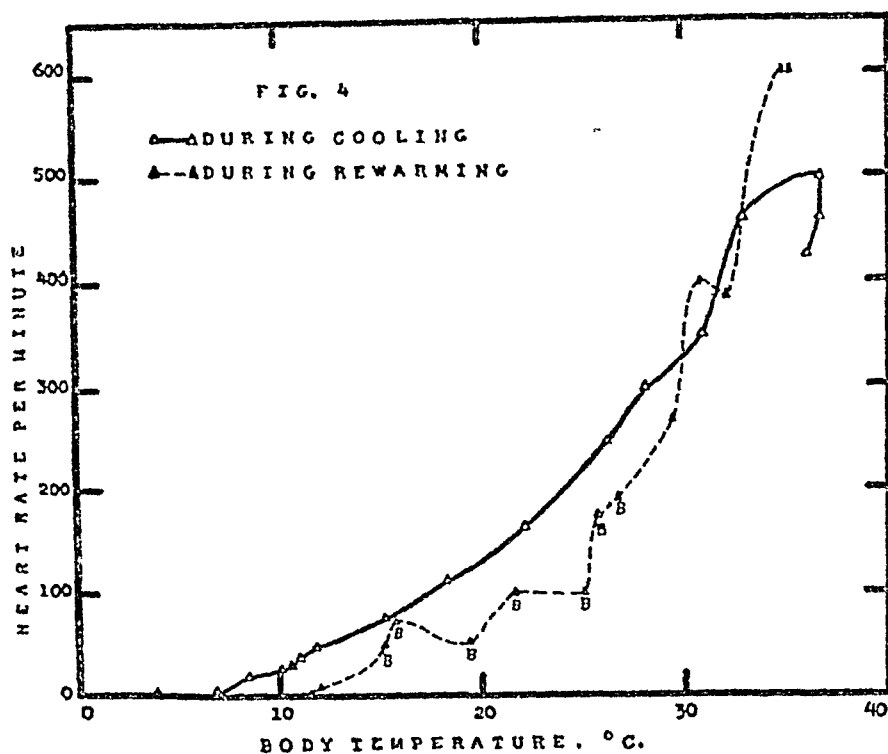


Fig. 4. HEART RATE AT DIFFERENT BODY TEMPERATURES, as a 9.5-day-old rat cools to  $2^{\circ}\text{C}$  and then is rewarmed. The points marked 'B' on the rewarming curve indicate abnormally slow conduction, or 2:1 or 3:1 blocks.

complete recovery of the animal. The only longer records of asystole occurred in animals which recovered their heart beats, but then died during the rewarming. When the animal is rewarmed, the heart beat reappears or increases within a few minutes and reaches the precooling level or higher.

In general, in the  $20^{\circ}$  experiments, the heart rate is slightly higher during the rising (recovering) body temperature than during the same body temperature as the animal cools. In the  $2^{\circ}$  experiments, however, just the reverse is true; the heart rate during rewarming is slower than during cooling, at the same body temperature (fig. 4). The explanation for this difference may be that in the  $2^{\circ}$  experiments, the electrocardiograms show conduction disturbances during the first part of the rewarming period, and these may account for the slower heart rates. It is also possible that

the heart may be warmer or cooler than the abdominal temperature which was the one measured.

Moreover, the heart is often faster in the final control period than during the initial control period; where the data were continued long enough, they indicate that this is followed by a slow fall to the normal level. This 'overshoot' is exemplified by figure 4. Possible explanations for the overshoot might be that heating is a more effective influence upon the cardiac apparatus than is cooling. Another explanation might be that the slightly anoxic cooled animal responds to cardiac-controlling reflexes more than does a warm, well-oxygenated animal. There is also the possibility that accumulated metabolites might stimulate the circulation during and after the warming.

The electrocardiographic data suggest a prognostic sign for the non-survival of the animal. Many of the rats developed abnormally slow conduction of the cardiac impulse, or even 2:1 or 3:1 blocks during the rewarming, but if the animal developed irregular QRS impulses, along with the block, apparently more serious injury to the auriculo-ventricular node and ventricular conduction system had occurred and the animal did not survive. Only irregularly can a sinus impulse reach the ventricular conduction system and musculature. Moreover, the ventricular pacemaker, the A-V node, is unable, apparently, to initiate its own rhythm effectively. All of the ventricular impulses then come from the sinus and the injury is apparently progressive. Fewer and fewer ventricular systoles occur and the animal dies within half an hour.

Occasionally, during rewarming of the animals which eventually survive, very slow conduction is followed by a 2:1 or 3:1 block as the parts of the heart become more normal physiologically. This seemingly paradoxical situation is due to the fact that, during the former condition, the sinus frequency is so slow that the ventricle is non-refractory by the time the slowly travelling impulse gets to it. This changes to a 2:1 or 3:1 block as the sinus frequency becomes faster, because conduction in the A-V node and bundle have not correspondingly improved, so the ventricle is still refractory after every second or third sinus impulse. This condition, however, soon disappears, leaving a physiologically normal heart as ventricular conduction improves. Occasionally, in some animals during rewarming, the ventricles developed a rhythm different from that of the auricles, but none of these animals survived this type of block.

It seems probable that the blocks produced in the rewarming of the infant rats were results of anoxia. Lewis's (13) description of the electrocardiograms of mature rats during progressive anoxia is identical with these results. His blocks readily disappeared when ventilation was restored if the asphyxia were not carried too far, and he believed that the obstruction was in the A-V node, because more peripheral parts of the conduction system could still conduct. When it is considered that tissues with a high metabolism are especially sensitive to anoxia, it seems reasonable that the presumably high glycogen-containing, high metabolizing tissues of the cardiac conduction system should be the first and perhaps only ones to be injured by anoxia.

Probably the cooling of the newborn rats produced too rapid a lowering of the metabolism of the whole body to cause anoxia. However, while the heart stops

beating, and oxygenated blood is therefore not being supplied to the body for over an hour, some body metabolism still exists and, as a result, the body and blood are becoming very slowly anoxic. When the animal is rather suddenly warmed, the metabolism suddenly increases and the heart and respiration may start too slowly at first to supply the sudden large needs for oxygen. The anoxia more severely interferes with the function of the cardiac conduction system than other tissues, so temporary heart blocks appear until the anoxia disappears and, if the anoxia has been severe enough, the injury is irreversible and the animal dies.

Effects of anoxia on dogs' hearts include congestion of the heart and veins with dilated auricles and pale contracted ventricles (14). The autopsies of the newborn cooled rats also showed these signs, all of which appear to be indications of the inability of the anoxic heart muscle to do its work. Alexander's (15) report of the German experiments on man mentions the finding of right heart and pulmonary engorgement in humans. Crismon (10) found the same in cooled adult rats and that the A-V blocks in his cooled adult rats disappeared during artificial ventilation. This may indicate that a deficient respiratory exchange adds to the deficient circulation. It is also possible that cold per se might cause enough injury, in some way, to produce these abnormalities. There were no detected cardiac abnormalities still existing at the end of the rewarming period when the surviving animal was removed from the respirometer.

In plotting heart rate, at 35°C., against age, it was noted that the heart rate is lower in the younger animals than it is in the older infants. Marcuse and Moore (16), who did not keep the body temperature constant, found the same thing in newborn rats. They point out that kittens and pigs have faster heart rates when very young and the rates decrease to the adult values. This phenomenon apparently depends on the species.

*Survival.* Of the 41 live rats tested, only 4 did not survive the experiment. Two died as a result of 5° exposures and 2 of lower temperatures. Permanent cessation of heart beat occurred in 3 during the rewarming period rather than during the actual cooling and in one, during a 3° exposure which was of much longer duration than any other, nine hours. There is no apparent correlation between non-survival and degree of exposure, except that all died at body temperatures of 7.5° or under. Many more animals, however, survived body temperatures as low as 2.5°C. There is also no correlation between death and rate of fall of body temperature, rise of body temperature on rewarming or age of the animal.

In exposures to 5° or lower, the majority of the animals showed cessation of heart beat followed by cessation of oxygen consumption, often for over an hour. Neither heart beat nor breathing, however, were required for survival of the animals. Most of the animals began to breathe and exhibit heart beats within a few minutes after the beginning of rewarming, the appearance of the heart beat again preceding the metabolic change. It is probable that these animals were not anoxic during the cooling period. It seems more likely that it is not until the rewarming period, when they are warmed enough to increase their metabolisms, that they experience any anoxia. The cardiac conduction system may be sensitive to anoxia in the newborn, though less than in the adult, and it may be irreparably damaged. This would

cause deficient circulation which would increase anoxia in the whole animal, as it is warmed, to an extent which would cause death.

The temperatures from which the newborn rats do not recover ( $7^{\circ}$  or below) differ markedly from those of adult rats ( $14^{\circ}$ – $16^{\circ}$ ). This difference may be attributed to several factors. The metabolism of the younger animal, per unit weight, is smaller, according to Himwich *et al.* (18); Negelein (17) reports that it also has potentially greater anaerobic ability. Therefore, anoxia would not as easily injure the brain of the newborn, which Himwich (18) believes is the limiting organ in tolerance to anoxia. That anaerobic metabolism is a method by which the young are able to withstand anoxia is indicated by the experiments of Enzmann and Pincus (19), which show that the rate of glycolysis in infant mice decreases with age, and Himwich's (18) finding that lactic acid production is higher in newborn rats, and iodoacetate and fluoride shorten the survival period in nitrogen; they conclude that anaerobic metabolism of carbohydrate is an important factor in the survival of anoxia in animals of different phases of development.

Fazekas, Alexander and Himwich (20) point out that newborn rats are less mature in development at birth than are dogs and guinea pigs and correlate this fact with the finding that newborn rats are able to survive anoxia longer than they. This tolerance, they point out, is lost with age. The animals become progressively more sensitive to oxygen lack, presumably because of the development of phylogenetically newer, more easily damaged parts of the brain. Newborn rats over periods of an hour or two seem comparable with cold-blooded animals in their ability to withstand extremely low body temperatures.

#### SUMMARY

Newborn white rats of 0 to 17 days of age were cooled to  $2^{\circ}$ ,  $5^{\circ}$ ,  $10^{\circ}$  or  $20^{\circ}\text{C}$ . in a respirometer filled with oxygen and immersed in a water bath. The immediate fall in the intraperitoneal temperature of the rats indicated that the rats were too immature to maintain body temperatures above environmental temperatures. At all temperatures below and including  $10^{\circ}\text{C}$ ., the exposures were too severe to elicit any but a transient increase in metabolism (as shown by oxygen consumption) and these transient and comparatively feeble protections against cold were manifested only in rats over three days of age. Ultimately, in all experiments on rats up to 10 days of age at  $3^{\circ}$  or lower, the metabolic rate reached zero and remained so for as long as 108 minutes, followed by complete recovery of the animal.

Electrocardiograms showed that the rate of the heart beat and of the conduction of the cardiac impulse decreased linearly with the decrease in body temperature, the heart beat disappearing when the body temperature reached between 9 and  $3.5^{\circ}\text{C}$ . The asystole lasted up to 82 minutes, followed by complete recovery of the animal and its acceptance by the mother rat. It was believed that the prognosis for non-survival of the 4 rats, which died out of the 41 exposed, was the finding of 2:1 or 3:1 block with irregular QRS impulses, suggesting serious injury to the A-V node and conduction system which apparently was irreversible. This lethal effect was manifested only in animals whose body temperatures were  $7^{\circ}$  or under and the anoxia during the rewarming period may have been the ultimate factor in survival or non-survival. These low lethal temperatures classify the newborn rat as a temporary poikilotherm.

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# TOLERANCE TO COLD AND ANOXIA IN INFANT RATS<sup>1</sup>

E. F. ADOLPH

*From the Department of Physiology, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

THE object of this investigation was to trace changes with age in the ability of infant animals to survive hypothermia. It has been known since the observations of Edwards (1) were published in 1824 that infant mammals survive the cooling of their bodies to lower temperatures than adults of the same species tolerate. The limits in temperature and in time that can be endured have not been ascertained for any species; the question arose whether indefinite poikilothermic existence was possible at certain ages.

Infants of various species have been known to survive lack of air or oxygen for considerable periods of time, ever since the observations of Robert Boyle (2) recorded in 1675 and the unrecorded experiences of obstetricians and stockbreeders of all centuries. What is the relation of the two tolerances, that to cold and that to anoxia? Do they disappear at the same age? If either or both tolerances could be preserved into adult life, considerable understanding of the animal's limitations toward cold and anoxia would be obtained.

The present investigation started from the observation that infant rats survived a body temperature of 2° to 5°C. for two hours when enclosed in an atmosphere of oxygen (3), but not when the body was immersed to the shoulders in water and the head was surrounded by air.

## PROCEDURES

The initial technique consisted in fastening rats to small boards by passing adhesive tape about each leg. Each infant rat had a thermocouple of fine nylon-wrapped wires (iron and constantan) inserted 1 to 3 cm. (depending on body size) into the colon, the wires being taped to the animal's tail. The potential differences, between colonic couple and reference couples to copper at 0°C., were balanced every six minutes with a recording potentiometer. Thermocouples were calibrated at the close of each experiment. After a period in room air the boards were clamped so that each of 5 or 6 rats of known ages were held in water of regulated temperature. Ordinarily the infant rats were immersed to the shoulders and neck; the trunk and limbs were covered, but the head was clear of the water even when the animal struggled. For a given experiment the stirred water was kept at a set temperature by a toluene-mercury regulator and relay connected to a compressor that supplied a refrigeration coil.

In testing the effects of various gaseous atmospheres, infant rats were placed in 250-ml. Erlenmeyer flasks, usually with thermocouples in their colons. The flasks were at first surrounded by water of 35°, later by water of a low temperature and finally by water of 35°. A stream of air or nitrogen or oxygen at room temperature passed continuously through each flask.

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Received for publication October 11, 1948.

<sup>1</sup> This investigation was aided by a contract between the Aeromedical Laboratory of the U. S. Air Forces and the University of Rochester. Important help in the experiments was rendered by D. Sherba and E. Guzman.

Survival was judged not by the maintenance of particular activities, for often all movements and reflexes disappeared, yet the animals revived. Instead, the criterion was that of recovery and continued viability when the rats were rewarmed at  $35^{\circ}$  with the head surrounded by air. Therefore, individuals (usually in pairs) were removed from cold at diverse times, the aim being to have some survive and some die. Lethal effects of cold and of anoxia turned out to differ enormously in infant rats of diverse ages. Therefore it became necessary to work out systematically, for a series of post-natal ages, the lengths of time for which given body temperatures could be endured.

Rates of body cooling differed greatly, depending on whether the infant rats were in water or in flasks (fig. 1). The course over which the colonic temperature decreased were only roughly similar in the two environments and in two temperatures.

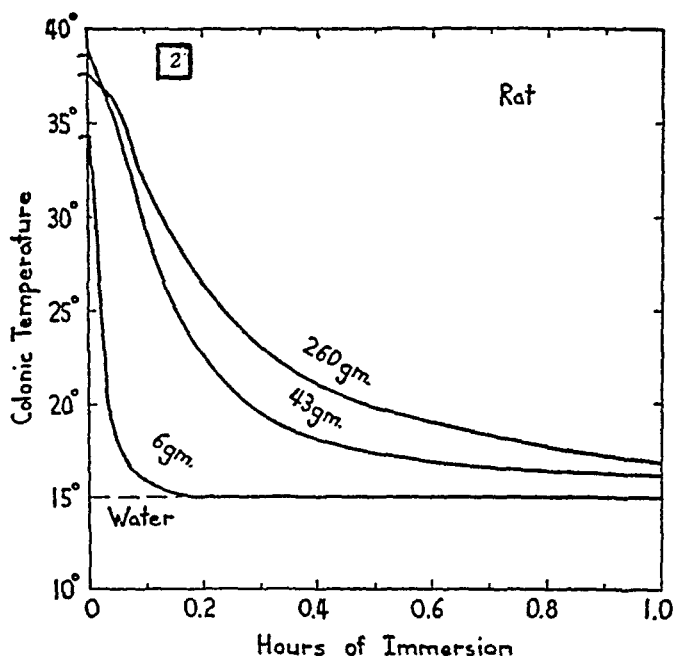
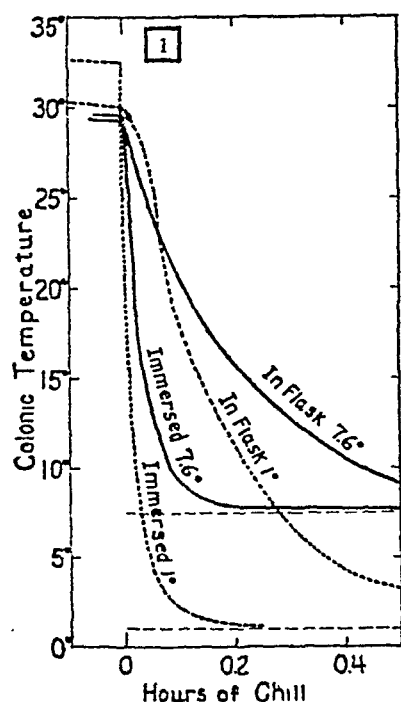


Fig. 1. COOLING CURVES OF INFANT RATS aged 9 days (wt. 15 gm.) under 4 conditions. In water of 2 different temperatures as indicated, rats were either immersed to the shoulders or were placed in a glass flask surrounded by water. Each curve represents the mean of 3 to 6 individuals simultaneously exposed, all of which survived treatment.

Fig. 2. COOLING CURVES FOR colonic temperatures in rats of 3 sizes that were immersed to the shoulders in water of  $15^{\circ}\text{C}.$  at zero time. Each curve represents the mean of 5 individuals simultaneously exposed. Their ages were 1 day, 27 days and about 200 days respectively.

For purposes of estimating the endurance times at diverse temperatures, the arbitrary rule was adopted of calling zero time when the colonic temperature passed  $15^{\circ}\text{C}.$  But in anoxic exposures, the rule was to start passing the nitrogen when the colonic temperature came within  $4^{\circ}\text{C}.$  of the water temperature and to call the initial passage of the gas zero time.

Rates of cooling differed with age and body mass (fig. 2). When the rats were immersed in water the courses of cooling were similar excepting for their time constants. However in air, rats down to 26 days of age could not be cooled more than a few degrees and those down to 10 days of age resisted cooling often for some hours so long as they were breathing. In nitrogen, however, the resistance to cooling disappeared. In general the colonic temperature ultimately came to lie within  $2^{\circ}\text{C}.$  of the water temperature; the younger the individual, the more nearly did the difference approach zero.

*Immersion in Water, Air around Head.* The endurance times varied enormously at diverse temperatures (fig. 3); a colonic temperature of  $2^{\circ}\text{C}.$  was endured

for only half an hour,  $10^{\circ}$  was endured for over two hours. Evidently precise knowledge of the temperatures of the vital tissues was the key to recognition of lethal conditions. Endurance times also varied with age, becoming shorter at any given body temperature. Probably the change was gradual as the animals aged, but in this series the data accumulated were only sufficient to show the marked diversity between two age groups (fig. 3).

Adult rats were unable to endure a deep temperature of  $14^{\circ}$  for even six minutes and usually died in two hours at  $15^{\circ}$ . Above that temperature long exposures appeared to be tolerated. Infant rats not only endured much lower temperature, but exposure time made a larger difference.

Since rate of cooling differed in animals of diverse sizes (and ages) (fig. 2), it could be supposed that slow cooling might yield other results in infant rats. In one test, the several individuals were placed in water of  $13^{\circ}$  and the temperature of the water was lowered during an hour's time to  $8^{\circ}$ . Survival was not extended by the

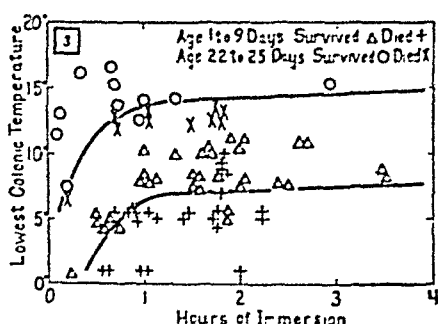


Fig. 3. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below  $15^{\circ}\text{C}$ . Each rat was immersed to the shoulders in water and the head was surrounded by *air*. Two age groups are represented; a curve for each group separates survivors from non-survivors.

slow cooling. The slow cooling characteristic of exposures in air was also little different (see next section).

Obviously the tissue temperature was of more lethal consequence than the external temperature. The endurance times in the graphs represent the lowest colonic temperatures attained for the period of time that the colonic temperature remained below  $15^{\circ}\text{C}$ . An inevitable feature of this form of comparison is that the younger the animal, the longer was the exposure to those low temperatures that approached the minimal, for it had cooled faster than older animals.

No evidence was found that rate of rewarming was of consequence. Actually, recovery depended upon artificial rewarming, especially in the youngest rats, for heat production was almost abolished in them (3) and heat could be received from room air only slowly. Infant rats often died if not fully rewarmed before they were returned to the mother; they were rejected by the mother more frequently when they were hypothermic.

Partial immersion allowed survival at colonic temperatures that were intolerable during immersion to the neck. Ordinarily the hind legs, tail and a small portion of the abdomen were then covered by the water. In such immersions the head and chest were measurably warmer than the colon. To ascertain this fact, thermo-

couples were sometimes placed in the upper esophagus. Moreover, the differences between the two regions increased with age; at 25 days the difference was only  $1^{\circ}$ ; in the adults a gradient up to  $4^{\circ}$  was found.

Initial experiments seemed to indicate that immersion of the chest had a more lethal effect than partial immersion. Subsequent tests showed that insufficient numbers of animals had been used. Any residual difference is now believed to be due to the fact that colonic temperatures did not represent the temperatures of vital regions during partial immersion. No factor other than temperature of chest or head has been demonstrated to influence the lethal conditions for infant rats breathing air.

Rats cooled below  $10^{\circ}\text{C}$ . did not breathe in any visible fashion. It seems likely that no air was pumped into the chest, but over periods greater than 1.5 hours, oxygen was indispensable, as will be shown later. Complete submergence was found to bring death in less than one hour; it was probable that water filled the respiratory passages during such treatment.

Other movements besides breathing were retarded as the infant rat cooled. Below  $8^{\circ}$  movements were rarely seen at all, even in response to prodding of the skin on the head. Total cessation of movement was no criterion of ultimate survival; this was the reason that the fate of each animal was ascertained by rewarming after a predetermined period of exposure to cold immersion.

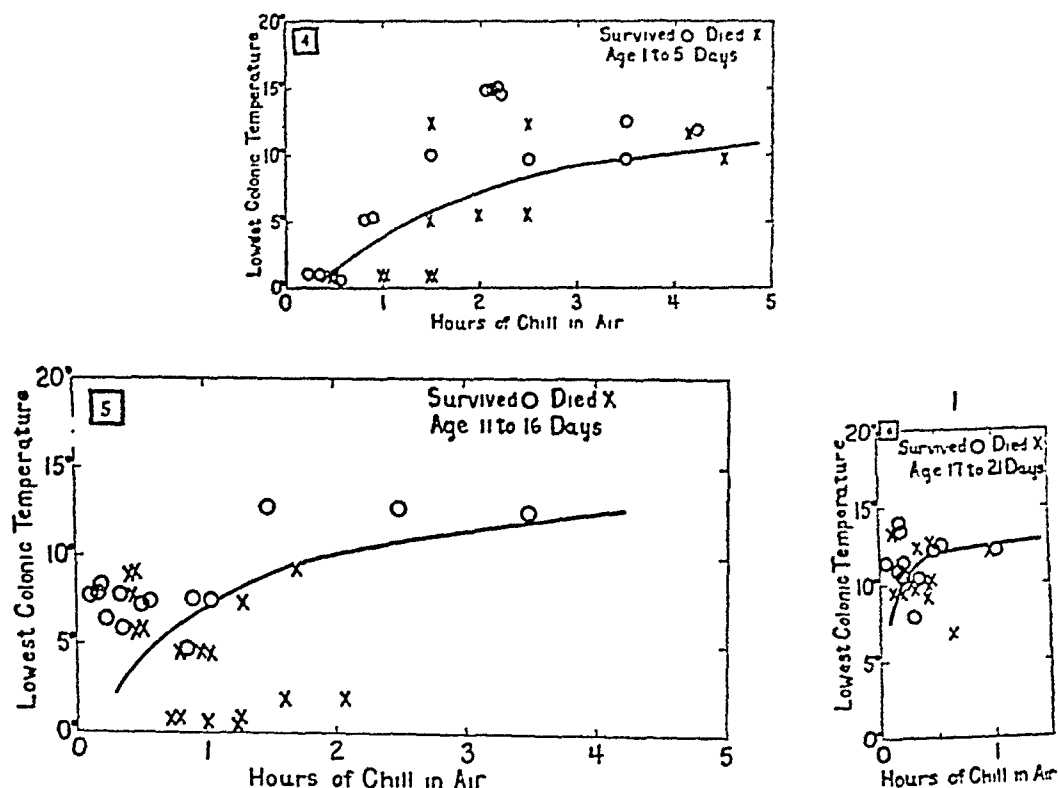
Movements often reappeared in one to two minutes of rewarming. Occasionally a rat that had been totally inactive in the cold required 10 to 20 minutes for recovery. Sometimes total inactivity occurred, the cold animal having a blue skin instead of the more usual pink. It is possible that blueness means cessation of heart beats, for electrocardiograms ceased to show cardiac conduction at some such times. In general, heart beats were no criterion of survival, for they might cease after cooling was over and often may resume after long cessation. Resumption of beats was also no prophecy of survival, since beats occasionally failed after returning for some period; this fact was ascertained by opening the chest as well as by recording the electrocardiograms.

Infant rats that died hypothermally revealed little that was unusual at autopsy. The lungs were pink or red instead of the white which characterized those that recovered. Probably more blood was contained in the pulmonary blood vessels during chilling; whether this fact was related to failure of survival in certain individuals is not known.

Infants of a few other species were tested by immersing them to the neck. Dog pups survived two hours with final colonic temperatures of  $10^{\circ}\text{C}$ . Two kittens, four days of age, survived 1.5 hours at colonic temperatures below  $15^{\circ}$  and down to  $7.2^{\circ}$  and  $9.2^{\circ}$ . Five others were killed during 1.8 hours between  $15^{\circ}$  and  $5.8^{\circ}$ . In another test, 2 two-day old guinea pigs were killed in only seven minutes of exposure between  $15^{\circ}$  and  $13^{\circ}\text{C}$ . of colonic temperature. That guinea pig infants are intolerant to cold was noted by Edwards. The same species is distinguished by the intolerance to anoxia in newborns (4). It is possible to say that guinea pigs are born in a state so mature that they have little tolerance either to cold or to anoxia; it remains to be ascertained whether they ever had either.

The above data reveal the marked effect of temperature that modifies the survival times of exposed rats. At 20 to 25 days of age the ability to endure temperatures less than  $15^{\circ}$  is completely lost. The possible constituents of this infantile ability to endure lower temperatures will be considered later in this report.

*Cooling in Air.* In this series the infant rats were kept in flasks surrounded by water of the desired temperature. At ages up to nine days they were cooled in the flasks; older ones were cooled initially by dipping them in the cold water itself; after which the low temperature persisted while they stayed in flasks. The data may



Figs. 4, 5, 6. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below  $15^{\circ}\text{C}$ . Each rat was kept in a flask surrounded by cold water; air slowly passed through the flask. Three age groups are represented; a curve is drawn in each group to separate survivors from non-survivors.

conveniently be referred to three age groups, though more exhaustive tests might show intermediate tolerance curves at intermediate ages.

The most complete determinations fell in ages one to five days (fig. 4). In this age the curve has nearly the same position as for the infants of similar age immersed in water (fig. 3). It is likely that the two curves would be identical in a larger number of tests.

The tolerance to cold was lost gradually, so that the endurance time in each temperature diminished between the ages of 1 and 20 days (figs. 5, 6). The endurable temperature for some one period of time became higher. The curves are roughly of a hyperbolic character, suggesting that cold and time together tend to yield a lethal product.

Variations of the exposure in air were made as follows: *a*) The infant rat was strapped to a small board as in the immersion tests, then covered by a thin rubber sack and immersed to the neck in water. *b*) It was placed in a flask and enclosed in a refrigerator. *c*) It was placed in a glass cylinder that was immersed in water while a constant stream of air flowed through the cylinder. In each variation only one point on the temperature-duration curve was established. None of the procedures

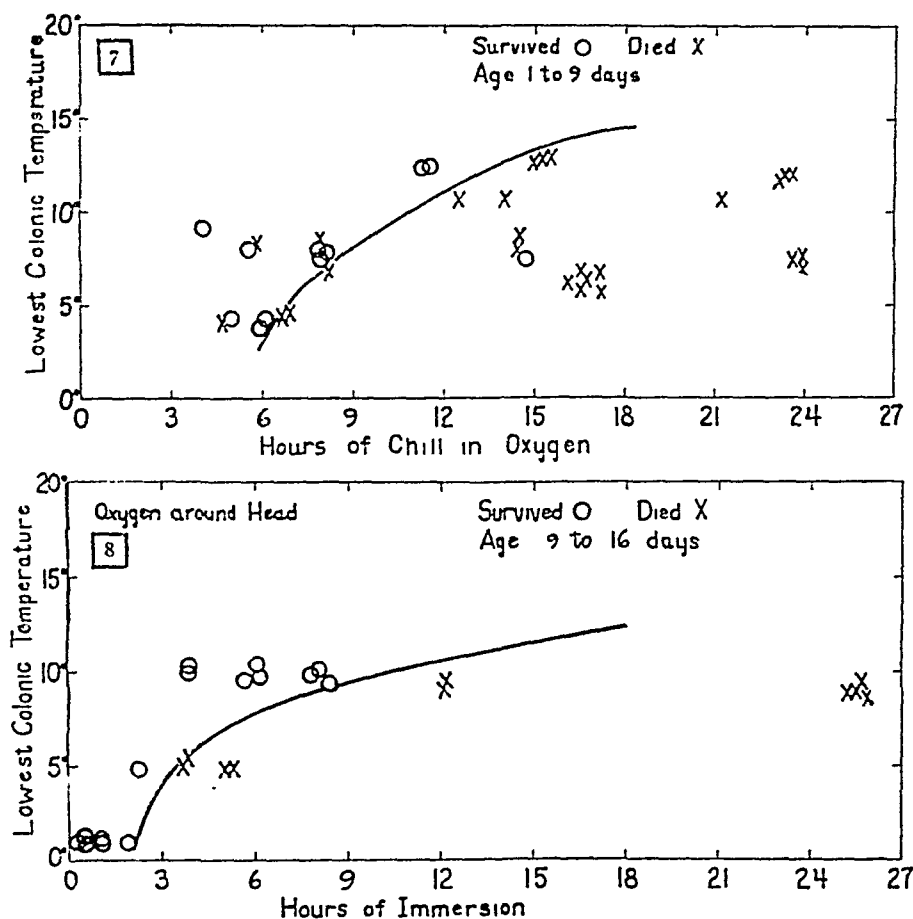


Fig. 7. COLONIC TEMPERATURES of infant rats in relation to the length of time those temperatures remained below  $15^{\circ}\text{C}$ . Each rat was kept in a flask surrounded by cold water; oxygen slowly passed through the flask.

Fig. 8. COLONIC TEMPERATURES in relation to time below  $15^{\circ}\text{C}$ . The infant rats were immersed to the shoulders in cold water and the head was covered by a cowl through which a stream of oxygen passed.

modified the results so long as lethality was referred to the tissue temperatures of the animals. Hence rate of cooling and of warming may also be said to be of no consequence.

The fact that infant rats endured lower tissue temperatures than adults was recorded by Wiesner (5) and Antoschkina (6). The time relations were not investigated heretofore and it came to us as a surprise to find that survival was limited in the lowest temperatures to half an hour, while at  $10^{\circ}$  endurance times up to five hours prevailed. In natural conditions the importance of nesting and the indispensability of periodic rewarming by the mother rat are apparent.

*Cooling in Oxygen.* The experiments of Fairfield (3) showed that infant rats in oxygen survived at least two hours at body temperatures of  $2^{\circ}\text{C}$ . and at least five hours at  $10^{\circ}$ . It seemed important to see what endurance limits could be obtained at these temperatures (fig. 7). Evidently cold could be endured for a much longer time in oxygen than in air. This fact indicated that tissues were being injured not merely by remaining at a low temperature for too long, but that a higher pressure of oxygen deferred the injury for some hours. At the same time, no oxygen consumption could be detected at  $2^{\circ}$  to  $5^{\circ}$  (3). In temperatures below  $6^{\circ}$  the heart usually ceased to beat (3); in such case a reserve of oxygen may exist in the animal when the oxygen has been supplied at high pressures during cooling. Oxygen may fill the lungs where it can be picked up by the blood whenever it is circulating; oxygen may be utilized by direct diffusion when the circulation has ceased.

No difference of survival was found between rats of one to five days of age and those of six to nine days of age in oxygen. The former were smaller and naked, while the latter had thicker skins as well as thicker torsos. If diffusion from the body surface were concerned in supplying oxygen, a marked difference would be expected.

*Immersion in water, oxygen around head.* At ages beyond nine days, infant rats were cooled more conveniently by immersion to the shoulders. When an atmosphere of oxygen was kept around the head (fig. 8) survival was much longer than in an atmosphere of air.

This result confirmed that of the previous series. It also showed that the aid of oxygen in prolonging survival extended to at least 16 days of age. Three tests made at 27 days of age at  $14^{\circ}$  showed that oxygen no longer protected rats from cold as compared with litter mates that had air around the head. The result also showed that the oxygen had its chief effect in the head or lung region and not over the general body surface. An effect was exerted whether the chest was breathing or not and whether the heart was beating or not.

*Cooling in Nitrogen.* The remarkable ability of the newborn mammal to survive without breathing or oxygen has been recognized for at least 300 years. William Harvey (7) in 1651 stated that, "I have often seen the human fetus extracted alive from the uterus when the mother has been dead some hours. I have also known the rabbit and hare to survive when extracted from the uterus of the dead mother". Similar observations upon dogs were made by Boyle; he also demonstrated that newborn cats would survive in rarefied air much longer than adults, though breathing and heart beat were suspended for some time. The history of the subject was recorded by Legallois (8) in 1824 who made important contributions of his own.

That temperature might affect the anoxic survival was found by Edwards (1) in 1824 who submersed newborn kittens in water of various temperatures. Movements persisted longest (up to 49 minutes) in water of  $20^{\circ}\text{C}$ .; their duration diminished sharply in water of both higher and lower temperatures ( $30^{\circ}$ ,  $42^{\circ}$ ;  $10^{\circ}$ ,  $0^{\circ}$ ).

It is well known that the tolerance to anoxia diminishes gradually with age, as was shown in various ways for rats by Bert (9), Reiss (10), Cameron (11), Fazekas *et al.* (12), Selle (13), Hiestand (14) and Cheymol (15). For the most part, however, these investigators neglected to control the animals' temperatures. Could it be that the tolerance to cold depended upon the tolerance to anoxia and was lost simultaneously with it?

Rats were therefore exposed to an atmosphere of nitrogen under specific conditions of body temperature. The infant rats were first cooled, then the air of the flasks which contained them was replaced by a rapid and steady stream of cylinder nitrogen. The length of time that infant rats could survive in absence of oxygen was, under the most favorable conditions, two hours (fig. 9). Tolerance times were greatest at about  $10^{\circ}$ . They diminished markedly and sharply at temperatures below  $5^{\circ}$ .

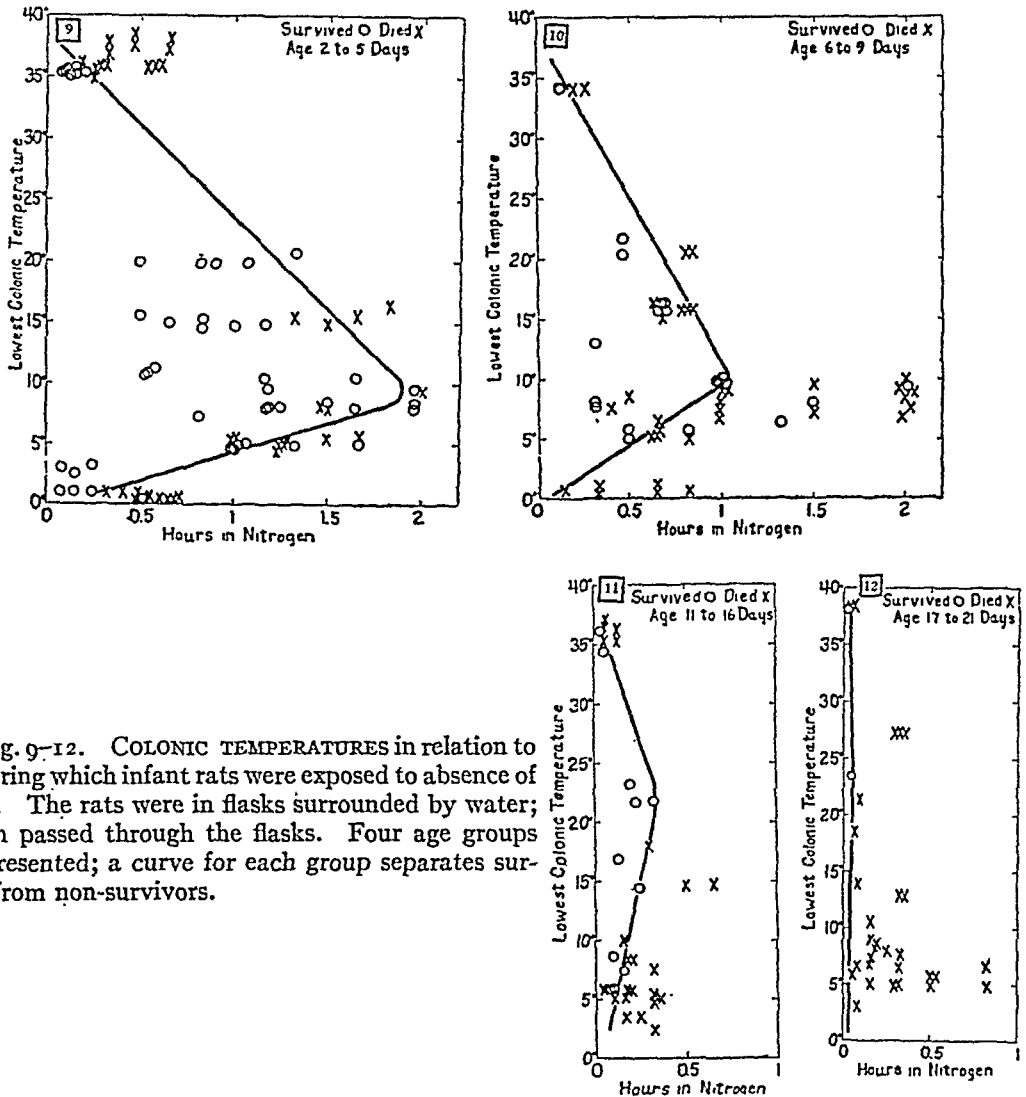


Fig. 9-12. COLONIC TEMPERATURES in relation to time during which infant rats were exposed to absence of oxygen. The rats were in flasks surrounded by water; nitrogen passed through the flasks. Four age groups are represented; a curve for each group separates survivors from non-survivors.

Tolerance times also diminished with age (fig. 10); so that by 11 days of age (fig. 11) the maximal time was only 0.3 hour and by 17 days (fig. 12) was that of the adult. The optimal temperature for survival in nitrogen increased from  $9^{\circ}$  in the newborn to somewhere between  $15^{\circ}$  and  $25^{\circ}$  at 12 days of age. In contrast to the tolerance in nitrogen, that in air was much greater at temperatures above  $5^{\circ}$  and lasted later in life, e.g. 11 to 13 days (fig. 13). In other words, tolerance to anoxia was largely lost before tolerance to cold was lost. The two appear to be independent in large part.

Oxygen aided survival even in temperatures below  $5^{\circ}$ , where air was scarcely of more aid than nitrogen and was effective over air at an age (16 days) at which anoxic survival had disappeared.



Further information about the effects of oxygen was gained from the following tests: *a)* Oxygen was injected into the peritoneal cavities of 7 infants of three days of age before they were cooled to  $5^{\circ}\text{C}$ . No appreciable difference occurred in survival when compared with an equal number of uninjected litter mates. *b)* Rubber collars were placed around the neck and sealed to it by rubber cement. Nitrogen was then passed to the head in 13 animals and to the trunk in 10 animals, while air irrigated the other compartment. In general at  $8^{\circ}$  those that had air around the head survived indefinitely longer than those with nitrogen around the head, even though no breathing could be detected. *c)* In 6 more infants an atmosphere of oxygen was kept around the trunk while nitrogen surrounded the head; they also survived in no greater proportion than when nitrogen bathed the whole body. It is concluded that oxygen is of no aid in survival except when it surrounds the head,

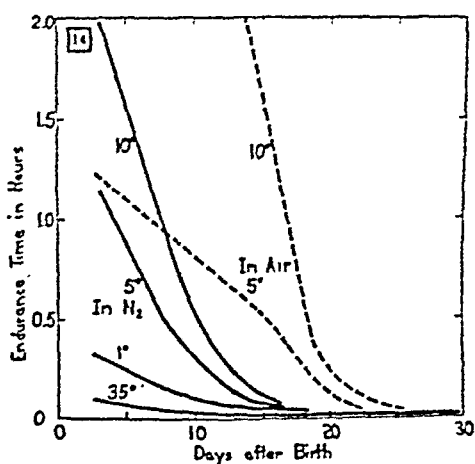
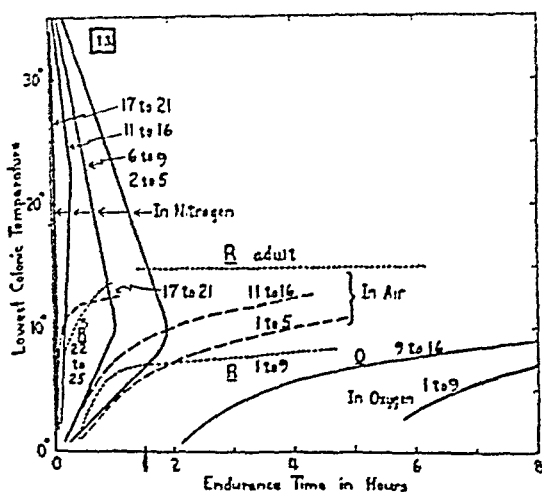


Fig. 13. COLONIC TEMPERATURES in relation to mean endurance or survival time in rats of various ages. The 12 curves are traced from preceding figures (adult is from next paper) and are fitted to common coordinates. Numbers refer to days of age; *R* refers to rats immersed in cold water to the shoulders, head surrounded by air; *O* refers to similar immersion, head surrounded by oxygen.

Fig. 14. ENDURANCE OR SURVIVAL TIME in nitrogen and in air in relation to age of infant rats. Each curve represents the body temperature indicated, as read off from figures 4-6 and 9-12.

whether breathing is going on or not. *d)* Glucose in high concentrations in tissues might prolong anoxic survival. In 14 tests glucose was injected intraperitoneally before 2- to 4-day-old rats were cooled to  $5^{\circ}$  or  $8^{\circ}$  and placed in an atmosphere of nitrogen. The same proportion survived as among uninjected rats. This result at low body temperatures does not agree with the increase in anoxic survival reported by Himwich *et al.* (16) and Selle (17) in infant rats that were presumably much warmer.

In sum, the survival of infant rats in the absence of oxygen proved to be highly sensitive to body temperature. Most of the anoxic survival disappeared in the first 10 days of life, while the survival in cold air lasted a second 10 days longer. Surrounding the body by air or oxygen while the head was in nitrogen did not aid nor did the injection of glucose.

*Comparisons of Three Variables.* The effects of various combinations of three

influences upon survival of infant rats have now been reported; they are age, body temperature and time. By transforming the coordinates, several additional features of the combined influences can be pictured. The interpolated results are available in figure 13.

When body temperatures were kept constant, the tolerance times diminished with age along the curves shown in figure 14. The curves have rather similar shapes whether the tolerance to low temperatures was tested in nitrogen or in air, but the time scales are very different. The ages at which tolerance was lost in the two atmospheres differ greatly. These curves represent the changes in those processes that limit the rat's tolerance. They are curves of growth in intolerance. The curves of figure 14 correct to constant temperature the similar curves that have been

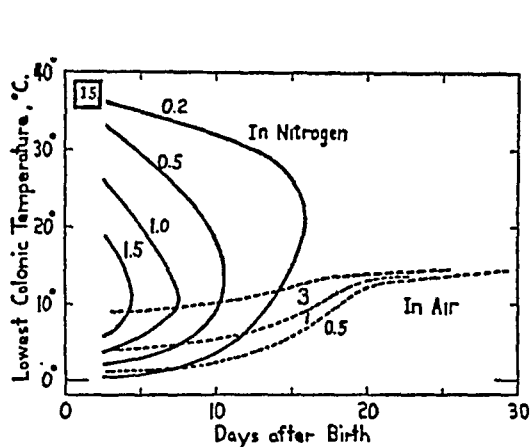
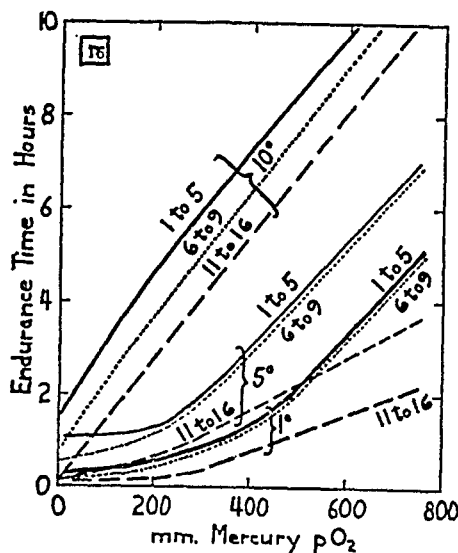


Fig. 15. RATS WITH VARIOUS BODY TEMPERATURES survive for the diverse periods of time indicated, when they are of the ages shown upon the abscissae. Curves are derived by transforming interpolations in previous figures.

Fig. 16. ENDURANCE TIMES of infant rats in relation to the oxygen pressure to which they are exposed at the several body temperatures indicated. Infants of 2 ages are represented. Curves are derived from previous figures.



constructed by those investigators who measured the tolerance times to anoxia at various ages without controlling the body temperature that prevailed. In their results the younger animals undoubtedly had lower temperatures than the older ones.

Figure 15 presents the contours for equal survival times at diverse ages and body temperatures. These contours define the safe conditions both in nitrogen (absence of oxygen) and in air. While during the first week of life, temperatures below 5° could be endured for about the same length of time regardless of the presence or absence of air, by the age of two weeks the tolerance to anoxia had been lost at all temperatures, leaving the tolerance to cold little impaired. Differentiation of the two tolerances is again evident.

Finally, interpolations at diverse pressures of oxygen are shown in figure 16. In 10° the hours of life added by each increment of oxygen pressure were about the same as in 1°, but advancing age eliminated the survival at low pressures before it

diminished greatly the time before death at high pressures. The results also suggest that survival may be greatly prolonged at oxygen pressures above one atmosphere.

#### COMMENT

Outstanding among the results of this investigation is the discovery that oxygen aids prolonged survival at body temperatures below  $12^{\circ}\text{C}$ . Breathing then has usually ceased. Thereafter it may be the supply of oxygen available in the lungs which is important. If so, the circulation of blood might transport the oxygen to other tissues. But below  $6^{\circ}$  there is no heart beat. Hence oxygen is reaching some critical tissues by diffusion through other tissues. The reserve of oxygen was shown to be either in the lungs or around the head. The fact that the reserve does not last indefinitely suggests the former. It might be inferred that in larger species of the same postnatal age, diffusion distances would be too great to allow such a large effect of oxygen. Whether this is so has not been ascertained.

Anaerobic survival of the young infant rat occurred at all temperatures. It was longest at  $10^{\circ}$ . Above that temperature it may be inferred that metabolism is slowest at the lower temperatures, so that a reserve lasts longer. Below that temperature some limitation prevails that has a negative temperature coefficient. It seems likely that this activity is the same temperature-sensitive one that also limits aerobic survival. In part this limitation is also oxygen-sensitive. It is clear, then, that cold has a lethal effect below  $10^{\circ}$  that is not limited by metabolic reserves, but perhaps represents the destruction of enzymic and other systems by low temperatures. The inference is drawn that cold is destructive in infant rats, at about the same temperature ( $8^{\circ}$  to  $10^{\circ}$ ) that it is destructive to peripheral tissues in adult rats and rabbits. The heart may be a limiting tissue in aerobic survival, but there is no evidence that such is usually the case in infant rats. The heart's activity is here divorced from survival. The heart very often resumes beating during rewarming, though this event alone does not foretell survival for other irreversible damage may have been suffered.

The minimal temperature for heart beats rises gradually with age, from  $6^{\circ}$  in the newborn to  $15^{\circ}$  in the 27-day-old rat. The parallelism of this rise with the rise in minimal aerobic survival suggests that they are interrelated.

Breathing movements serve as signs of life at temperatures above  $15^{\circ}$ . Survival is certain as long as gasping (jaw movements) continues. Observers (13) distinguish an aerobic series of gasps from a subsequent, less frequent, anoxic series. After the last gasp, survival is no longer possible, but at temperatures below  $15^{\circ}$ , breathing movements are usually absent. No longer do they serve as signs of survival; they are suspended in cold and are readily resumed during rewarming. Occasionally artificial breathing may be imposed with probable advantage, but in most tests here it has been omitted. In certain instances foamy fluid issued from the mouth when the chest was squeezed and thereafter gasping was resumed. Hence it is always possible that mechanical obstruction is sufficient to influence spontaneous activities that would otherwise secure survival. Other movements such as pendular limb movements, and responses to mechanical irritation, occasionally serve as signs of life but cannot be counted on. They may well depend upon portions of the neuromuscular system that do not determine ultimate survival.

At times skin color was thought to indicate viability. The majority of chilled infant rats had bright pink surfaces, but some were blue or dusky. The pink ones usually recovered as soon as rewarmed, but occasionally not at all. The blue ones rarely recovered promptly, but in some instances resumed gasping, then breathing, then other movements, after many minutes of rewarming. There seems, therefore, to be no combination of signs by which the capacity to recover from chilling can be foretold.

#### SUMMARY

Infant rats of ages up to 27 days were cooled to known body temperatures, either without or with immersion in water. After various times of exposure they were rewarmed to ascertain survival; all signs of life may be absent during hypothermia. In an atmosphere of nitrogen, newborn rats survived for two hours at 10°C., for shorter times at lower or higher temperatures. The presence of air enhanced survival above 5° and an atmosphere of oxygen prolonged it greatly at all temperatures up to 12°. With increasing age the tolerance to anoxia was lost before the tolerance to cold. This is evidence that these two remarkable tolerances do not depend upon the same critical process.

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# LETHAL LIMITS OF COLD IMMERSION IN ADULT RATS<sup>1</sup>

E. F. ADOLPH

*From the Department of Physiology, University of Rochester School of Medicine and Dentistry*

ROCHESTER, NEW YORK

THE object of this investigation was to ascertain what conditions limit an animal's endurance of immersion in cold water. Is the limit set by the temperature to which the body or certain tissues of it are chilled? Can the limit be changed by previous treatments? How variable are the endurances and susceptibilities among individuals?

Methods of testing tolerances to cold are numerous and diverse. Local or general cooling can be employed; any of a score of responses can be observed. Each of the responses represents a particular combination of functions and properties of the organism. No one test of resistance to cold will assay all the factors that may play a part in tolerance. The survival test here used, however, constitutes a practical over-all test.

Studies of cold *immersion* have been performed by other investigators; few (1, 2, 3) have employed small mammals. One of the questions to be answered here is whether or not cooling by immersion is intrinsically different from any other cooling. Only limited studies of hypothermia have in the past been made without anesthesia; it is important to know whether the anesthetics used by others influenced the extremes of temperatures that could be endured.

## PROCEDURES

In the present work, albino rats were restrained by taping their legs to wooden holders. Thereafter they were immersed, usually to the shoulders, in water that was (in most experiments) of a temperature that could be endured by some individuals for two hours. Thereafter the rats were withdrawn from the water and allowed to warm in room air. Only those that survived for an indefinite period thereafter were considered to have endured the hypothermic experience. These conditions of cooling were chosen because they paralleled those under which men are placed during immersion in open oceans.

To measure the temperatures attained within the body, thermojunctions were inserted 5 cm. within the colon. The wires leading from the junction were held in place by taping them to the tail. Sometimes additional junctions were placed in the esophagus; in this case the wires were encased in metal tubes that prevented the rats from chewing the wires. The wires used (30-gauge) were nylon-wrapped iron and constantan; both were contained in a flexible bundle only 1 mm. in outside diameter. Junctions were soldered and covered with deKhotinsky cement. In each circuit two cold junctions (to copper) were surrounded by ice and water; the potentials in each thermojunction circuit were automatically balanced and registered by a recording electronic potentiometer. Successive readings were accurate to  $\pm 0.1^{\circ}\text{C}.$ ; however, during 24 hours the calibration changed by  $\pm 0.3^{\circ}$  on the average. Hence, for most purposes, calibrations were made daily. As the calibration shifted, however, the increment of potential per degree of temperature did not change.

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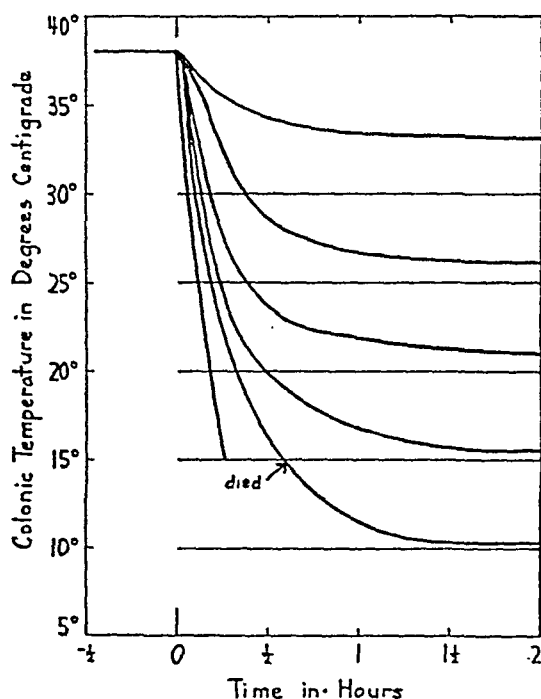
Received for publication October 11, 1948.

<sup>1</sup> The study was aided by a contract between the Aeromedical Laboratory, U. S. Air Forces, and the University of Rochester. Help in the experiments was given by W. B. Payne, D. Sherba and E. Guzman.

*Course of Cooling.* Colonic temperatures diminished with time in a highly reproducible fashion (fig. 1). A slight lag, lasting less than two minutes, was followed by a precipitous fall and by a final approach to an asymptote which approximated the water temperature. In rats of 200-gm. body weight, immersed to the shoulders, half the difference between initial colonic temperature and water temperature was traversed in about 13 minutes, regardless of the water temperature, provided the latter did not exceed 20°C.

The final difference of temperature between colon and medium was largest when the body's tissues were at 34° (fig. 2). Evidently this gradient in a warm rat can amount to 4°C. in a tissue thickness of only 2 cm. or less. This large gradient resulted from at least two factors: a rapid production of heat which was greater than at neutral or usual body temperatures (38°) and great internal insulation, secured by

Fig. 1. COURSES OF COLONIC TEMPERATURE in rats of 200 to 260-gm. body weight when immersed to the shoulders in water of diverse temperatures. Each curve is the mean of 5 individuals exposed at one time.



minimalization of blood flow. These factors were illustrated by comparing the cooling curves of living rats and dead rats (fig. 2). In contrast, when the rats were in water of 37° the heat production was low, the insulation being dissipated by rapid circulation of blood to the body surface. At any one time after immersion, colonic temperatures tended to be linearly related to water temperatures (fig. 3). Compensations successfully combatted this tendency only in the region of 30° water temperature. In water of 20° and below, the difference of temperature between colon and medium at the end of two hours was less than 1°C. Esophageal thermocouples indicated that the chest region also had similar temperatures (table 1).

When rats were immersed only to the hips, cooling was considerably slower (fig. 4). Moreover, the anterior regions of the body could now be considerably warmer than the posterior (table 1), by as much as 4°C. Seemingly the blood flow to the cooled posterior regions diminished to a point where little heat was carried to them.

When the rats were enclosed in rubber jackets that were held some distance from the body surface, cooling was very greatly delayed (fig. 4). The insulating effect of an air layer next to the skin is well known and its remarkable protection was here illustrated. In  $12^{\circ}$  water the rat immersed to the shoulders died in less than one hour;

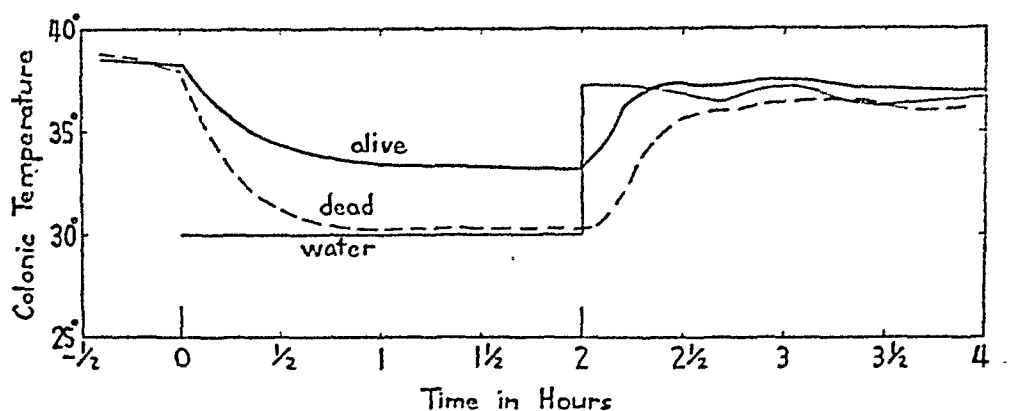


Fig. 2. COURSES OF COLONIC TEMPERATURES in rats of 225 gm. each, immersed in water of  $30^{\circ}$  and then transferred to water of about  $37^{\circ}$ . The solid curve represents the mean of 4 living rats the dash curve the mean of 2 rats previously killed by placing them in nitrogen for 3 min.

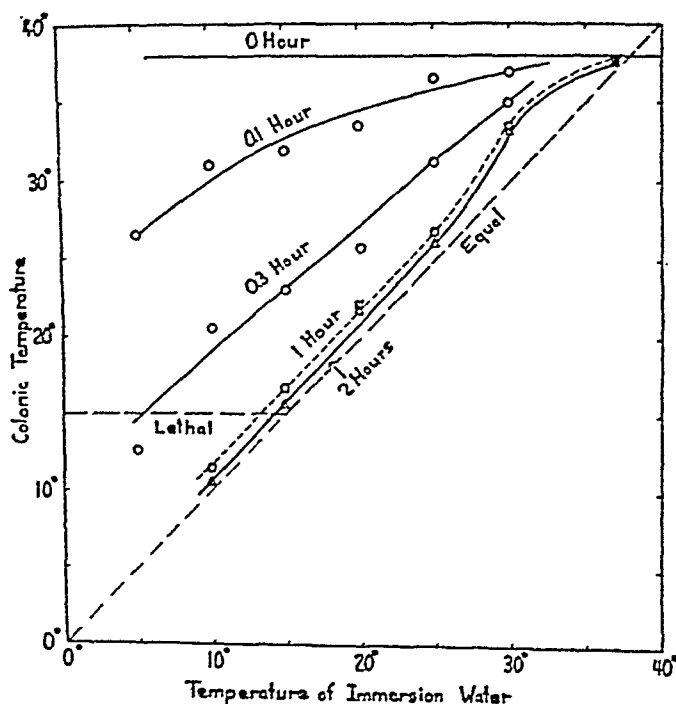


Fig. 3. COLONIC TEMPERATURES in relation to water temperatures surrounding the rats. Points were read off from fig. 1. Survival is usual within the area enclosed by dash lines.

the rat immersed to the hips might survive for 8 hours; and the rat in a jacket would hardly be in danger of death.

**Lethality.** Each standard test of ability to survive was conducted by immersing to the shoulders 5 rats, of about 200 gm. each, for two hours in a large water bath that was held constant within  $\pm 0.1^{\circ}\text{C}$ . Subsequent rewarming was usually 'spontaneous' in air of about  $25^{\circ}\text{C}$ . and after three hours of it the animals were unbound and returned to cages. Those alive at the end of 20 or more hours were found to survive indefinitely.

The mortality curve was established on 90 control rats (fig. 5), about half of which died in two hours. By interpolation, the median lethal temperature of the water was 14.8°C. The lethal colonic temperature cannot be established quite so

TABLE 1. SIMULTANEOUS TEMPERATURES IN ESOPHAGUS AND IN COLON OF RATS COOLING WHILE IMMERSED IN WATER

IMMERSION	WATER TEMP.	DURATION	FINAL COLONIC T.	FINAL ESOPH. T.	EXPIRED AT	COLONIC T. THEN	ESOPH. T. THEN
	°C.	hr.	°C.	°C.	hr.	°C.	°C.
in jacket	12.0	6.2	12.4	15.0	3.2	15.6	17.5
to hips	10.0	5.0	11.7	15.7	night		
	10.0	5.0	11.9	14.8	night		
to shoulders	20.0 to 11.8	5.0	12.0	12.3	2.5	14.5	15.2
	13.4 to 11.8	2.0	11.9	11.9	0.9	13.8	13.1
to hips	12.0	5.0	12.1	14.9	3.1	12.6	14.9
	12.0	5.0	12.2	15.0	5.2	12.4	15.2
	10.0	3.0	10.9	14.7	1.7	10.9	14.9
	10.0	3.0	12.5	16.2			
	10.0	3.0	11.9	16.9			
	10.0	3.0	10.4	11.8	2.3	10.5	13.3

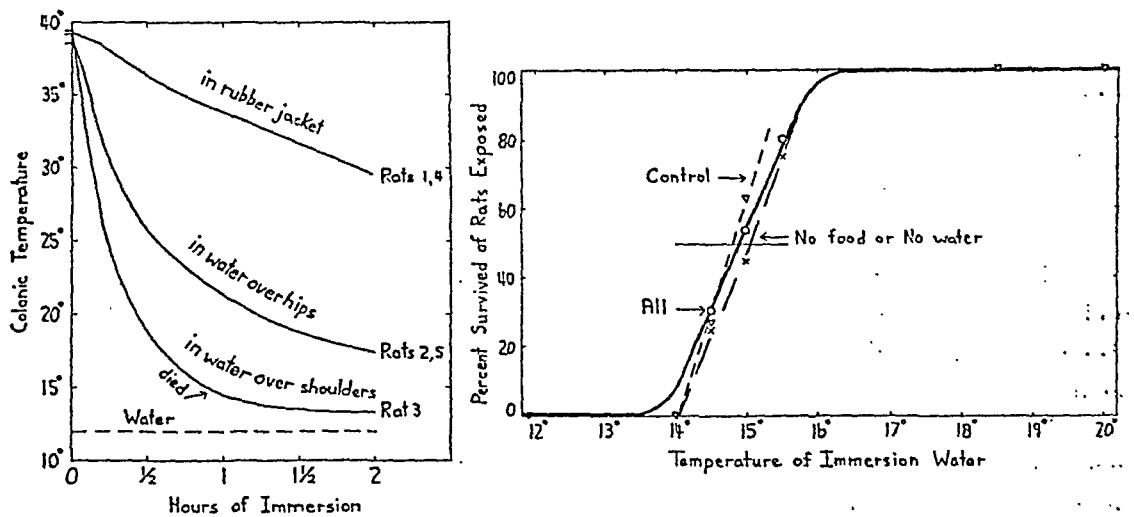


Fig. 4 (left). COURSES OF COOLING in rats treated in 3 ways in one water bath at 15°C.

Fig. 5 (right). FRACTION OF RATS that survive when for 2 hours they are immersed to the shoulders in water of the temperatures indicated. The numbers tested are: 15 at 14.0°, 35 at 14.5° and 50 at 15.0°, 5-10 at each other point. Some of the treatments, as in table 2, are indicated to show that subsequent differences in survival are not significant.

accurately, since during these tests the calibrations of the thermojunctions varied by as much as 0.3°C. greater than the water temperature at the end of two hours immersion to the shoulders, making the mean lethal colonic temperature 15.1°C. A roughly similar lethal temperature was reported by Hamilton (4, 5) for unanesthetized rats that were cooled by immobilization in cold air.



In rats so immersed, breathing became uncertain, irregular and slow as the animals cooled. It could not be affirmed with certainty, however, that breathing wholly ceased in any individuals that survived. Heart beats continued in all survivals in which electrocardiograms were taken. Reflex movements of the head, and wink responses to mechanical irritation of the eye-lids, persisted in nearly all individuals that recovered. However, in 13 rats it was specifically ascertained that wink reflexes disappeared, yet the individuals recovered later. The time of immersion at which the wink reflex disappeared is shown in figure 6 for each individual that succumbed of those represented in figure 5. At each of four temperatures the median times for this 'death' are indicated. In water of  $14.5^{\circ}$ , 30 per cent of the animals recovered and survived; in  $15.0^{\circ}$ , 65 per cent survived.

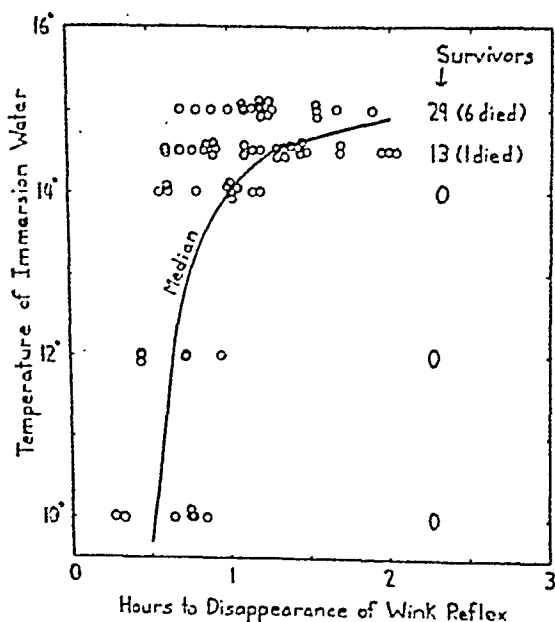


Fig. 6. TEMPERATURE of immersion water in relation to time before loss of wink reflex. At the end of 2 hr. rats were removed from the cold water and allowed to warm slowly; those that had not lost the reflex are designated as survivors, but of them 7 more died within 24 hr.

The body weights of the animals that succumbed before the median time were no less than those of the animals that lasted longer. However, other rats of less than 100-gm. body weight succumbed sooner; they are not included in this series. In such small individuals the cooling was more rapid, and slightly greater.

*Variability.* The mortality curve of figure 6 indicates that some individuals were more tolerant than others. Differentiation of this curve would show a frequency distribution of the lethal temperatures. Two thirds of the individuals would be included in a range of lethal temperatures within  $\pm 0.5^{\circ}$  of the median. This therefore is the standard deviation among lethal water temperatures for two-hour exposures. That the variability of lethal effects was in time as well as in temperature was shown by the disappearance of reflexes (fig. 6). Once the reflex wink had faded, survival had become improbable.

Some of the variability was within the individual. In one instance a rat that survived in water of  $14.5^{\circ}$  succumbed a week later during immersion in water of  $15.0^{\circ}$ . But no regular effect of previous subjection to cold immersion was found, providing several days intervened between exposures. Part of the variability was prob-

ably in the treatment of the rats. It was not possible to equalize the exact extents of immersion, the bodily positions (which are concerned in struggling) and the stirring of the water. None of these factors was clearly proven to matter, however.

Detailed study of single individuals that were cooled while surrounded by rubber jackets showed marked diversities of pulse rates (ascertained from electrocardiograms) at any colonic temperature that might be chosen. The heart was seen to beat at a colonic temperature as low as  $13.8^{\circ}$ , with subsequent recoveries of the animals after the wink reflex had temporarily disappeared. The heart could beat as infrequently as 20 times per minute (400 beats/min. at  $38^{\circ}$ ). It could beat irregularly, often with partial block, and still recover, but it has not been seen to recover after cessation of as long as 10 seconds. During the rapid cooling and re-warming of these tests, the temperature of the chest might differ appreciably from that of the colon.

*Various Rates and Durations of Cooling.* The rates of cooling of rats were varied by *a*) using water of diverse temperatures from  $5^{\circ}$  to  $15^{\circ}$ ; *b*) immersing the rats to diverse extents; *c*) surrounding the animals with rubber jackets; and *d*) gradually cooling the water in which the rats were immersed to the shoulders. From all these experiments the conclusion was drawn that lethal injury resulted whenever the temperature of the anterior half of the body had decreased to  $14^{\circ}$  to  $15^{\circ}$ . The period of time (up to 6 hr.) during which the body remained at this temperature mattered chiefly in the fact that disappearance of reflexes and other functions caught up with more individuals. For the animals immersed only at the posterior end, the colonic temperatures could be 3 to  $4^{\circ}\text{C.}$  lower than the esophageal temperatures (table 1). No attempt was made to find whether the chest or the head was the critical location for the low temperature.

Whether the lethal damage occurred in a particular tissue, or in several, was uncertain. The heart beat, the breathing and the eye-wink reflex all slowed as the temperature of the animal fell and tended to reach zero at  $13^{\circ}$  to  $15^{\circ}\text{C.}$  While the continuance of these functions could not always be taken as guaranteeing survival, nor their cessation as preventing it, the functions were probably critical ones. Similarly, it is not implied that colonic temperature was the only factor determining survival. This report is not specifically concerned with identifying the modes of death, but with establishing a reproducible test of lethality in cold, against which the protective power of diverse procedures might be tested.

Present evidence points to the conclusion that the *duration* of the lethal tissue temperature ( $15.1^{\circ} \pm 0.5^{\circ}$ ) was of little importance. In five tests, individuals were rapidly cooled and then quickly rewarmed, so that their colonic temperatures dipped below  $15.0^{\circ}$  for only 6 to 18 minutes. However, two of them were killed just as were individuals maintained at  $14.7^{\circ}$  for two hours. On the other hand, continuance of the cold immersion up to five hours at  $15^{\circ}$  was not significantly more lethal than for two hours. However, for 12 hours, water of  $16^{\circ}$  came to kill the rats immersed in it.

#### *Possible Influences upon Survival in Hypothermia.*

*a) Age.* Rats of all ages above 26 days died at the same average body temperatures when immersed in cold water. In the series of experiments so far quoted, animals ranged from  $1\frac{1}{2}$  to 12

months in age, and 103 to 360 grams in weight. Other individuals of 26 and 27 days of age (40-49 grams) were tested for 2 hours; all in 14° water died; three-fifths survived in 15° water. Hence from 26 days upward no differences due to age or size were found, providing the chest temperatures attained were brought close to the water temperatures. Only rats in earlier infancy survived lower body temperatures.

*b) Sex.* Equal numbers of males and females were used in the main series. No difference of mortality was found between them.

*c) Season.* No differences of lethal temperatures were found among series of tests done in each quarter of the calendar year.

*d) Rate of rewarming.* Most of the rats exposed to cold were allowed to rewarm in air of 25°. More rapid rewarming occurred in air of higher temperature usually 30°; still more rapid in water of 35°. Survival of the rats differed only to the small extent that some were incompletely rewarmed before they were returned to their food cages, causing delayed death (see below).

*e) Deprivation of food.* Individuals were kept without food but with water for 2, 3, or 4 days before they were chilled. The difference in survival was scarcely significant (fig. 5 and table 2); and survival was less than in controls, a result opposite to that noted by Fuhrman and Crismon (6) in anesthetized rats.

*f) Deprivation of water* for similar periods of time likewise did not favor survival. It may be remarked that privation of either food or water induced self-denial of the other item of intake (7).

*g) Glucose* was administered, usually 1.5 gm. by stomach tube, to 11 rats. The proportion of them that survived chilling was no different from that of simultaneous controls (table 2). In anesthetized rats, administration of glucose just before cooling upon chilled metal, irregularly delayed the stoppage of heart and breathing movements (6).

*h) Adrenal cortical hormone* (cortin) was injected subcutaneously (0.5 or 1.0 ml. Parke Davis), with no deviation in lethal effect from that of the controls. This result also contrasts with that of the above investigators.

*i) Oxygen around head.* Rats that were being cooled by immersion to the shoulders had their heads covered by a rubber cowl, into which passed a continuous stream of oxygen. By this means their lungs were left filled with an atmosphere of oxygen whenever they stopped breathing. In contrast to the positive result in infant rats, no greater survival resulted here (table 2).

*j) Digitalis.* Equal numbers of tests were made after intraperitoneal injection (at 20-60 minutes before chilling) of each of the following: 0.67 ml. digalen, 0.17 ml. digalen, 0.60 ml. cedilanide (or lanatoside C). Mortality in water of 14.5° did not decrease, contrary to expectation from the results of Crismon and Elliott (8) but not from those of Barbour *et al.* (9).

*k) Acclimatization.* Rats were acclimatized to cold by placing them with adequate food and water in air of about 7°C. for periods of 11, 17 or 30 days. If any treatment would make animals more tolerant to cold, this might well be it. But, no more survival was found than in control rats. Acclimatization may be considered as occurring in two possible ways: by change of resistance to cooling (by more production of heat or by less loss of heat or by both) and by change of tolerance of low temperatures within the tissues. In the present tests all resistance to cooling is rapidly overcome. Tolerance of cold is alone being tested; such tolerance has not been successfully modified.

The test of survival here employed is a sensitive one. In all cases the colonic temperatures of the treated rats did not differ by more than a few tenths of a degree from those of the control rats. However, the colonic temperatures that could be tolerated at the end of two hours of immersion up to the shoulders varied by a standard deviation of  $\pm 0.5^{\circ}\text{C}$ . No treatment was found that lowered the median lethal temperature by even  $0.2^{\circ}\text{C}$ . Hence it may be concluded that the temperature, at which the tissues that limit a rat's survival are injured, is fixed to such an extent that it has not been modified by any of the influences tried.

The hibernating individual and the infant are the two kinds of mammals known whose tissues come to survive temperatures that are otherwise lethal. The hiber-

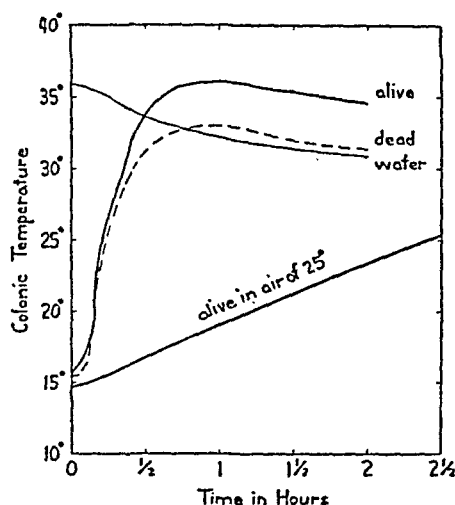
nating animal continues its breathing and blood-circulation at very low tissue temperatures ( $1^{\circ}\text{C}.$ ); only the infant survives (for a couple of hours) without either.

*Delayed Death.* In 21 rats exposed to cold apparent recovery began, only to be followed some hours later by death of the animals. These rats generally failed to regain their usual body temperatures 'spontaneously'; artificial warming would probably have saved them. Such deaths occurred occasionally in two or four hours after rewarming began, but more often happened during the night after an immersion. None occurred more than 24 hours after emersion.

TABLE 2. MORTALITY OF TREATED AND CONTROL RATS IMMERSSED TO THE SHOULDERS FOR TWO HOURS IN WATER OF CRITICAL TEMPERATURE

TREATMENT	WATER TEMP.	NO. TREATED	PER CENT SURVIVED	NO. OF SIMULTANEOUS CONTROLS	PER CENT SURVIVED
e. No food.....	$15.0^{\circ}$	14	43	9	89
f. No water.....	$15.0^{\circ}$	12	50	6	100
g. Glucose.....	$14.5^{\circ}$	11	55	8	50
h. Cortin.....	$14.5^{\circ}$	6	50	4	50
i. Oxygen at head.....	$14.5^{\circ}$	10	20	5	80
j. Digitalis.....	$14.5^{\circ}$	18	33	12	58
k. Acclimatized.....	$14.5^{\circ}$	15	47	10	60

Fig. 7. COURSES OF REWARMING in room air and in warm water. Each curve represents the mean of 2-5 individuals.



It seems probable that the lethal damage was inflicted during the period of low body temperature. No observed signs allowed prediction of the imminence of death; on the average these animals warmed more slowly and showed poorly coordinated locomotion. Where the lethal injury lay is unknown, but the central nervous tissue is naturally suspected. This form of delayed death was less critical than that found in heat stroke (10). In the latter, rats died up to two hours after they were cooled from a critical hyperthermia produced in a hot atmosphere, but both heat injury and cold injury may become irreversible some time before circulation and breathing actually cease. Delayed deaths have also been observed during 'recoveries' from food privation and from water privation.

At autopsy, rats that were killed in acute hypothermia or that died after hours of delay showed but little uniform abnormality. The lungs were congested to varying degrees; the congestion appeared to be uniform over the lungs of most individuals, but occasionally involved localized hemorrhages. Consolidation of the lungs has been sometimes observed but may have preceded the hypothermia.

*Course of Rewarming.* The progress of rewarming was surprisingly slow in rats. On the average they required 52 minutes to warm by the first  $2^{\circ}\text{C}$ . after emersion into air of  $25^{\circ}$ . The rewarming was steady (fig. 7) and became somewhat more rapid as the animals became warmer and resumed movements. The colonic temperatures of dead individuals leveled off, with the air temperature as asymptote. One fourth of the recovery of the 200-gm. living rat (from the minimal body temperature to the body temperature that preceded immersion) required 82 minutes, one half of it required 145 minutes. The latter could be reduced to 20 minutes by immersing the rat at once in water of  $30^{\circ}\text{C}$ .

The rate of rewarming in air manifested the animal's inherent abilities to recover after its body temperature had been disturbed. Evidently a rat cooled to below  $20^{\circ}\text{C}$ . was almost paralyzed in its recovery. Heat production was exceedingly small, reflexes had partially disappeared, circulation of blood was slow. Even when free movements had been recovered, shivering was small; rats do not have violent shivering as a part of their armamentarium of compensation for cold.

The chief effect of artificial rewarming was that it prevented the deep temperature from falling still lower after the animal was removed from water that was colder than the colon; when the rat had been quickly cooled in water of  $5^{\circ}$  to  $10^{\circ}$ , cooling by an additional degree or two could occur after emersion. In such a case rapid rewarming might be of vital consequence, but once temperature equilibrium had been established between body and water, the temperature did not diminish after the animal was emersed, for no evaporative cooling then occurred. Evaporation was already minimal, because the body was cooler than the air and the air was nearly saturated with moisture; the body might even gain heat by condensation of moisture.

#### COMMENT

The above experiments measure the lethal effects of cold in terms of the temperatures to which tissues are subjected and from which they can recover. It is shown that the mature rat is killed whenever the temperature of its head and chest is decreased to  $15.1^{\circ}\text{C} \pm 0.5^{\circ}$  (standard deviation). The variability of tolerance in the tissues of individuals is therefore small indeed.

No method was found of modifying the lethal temperature. Plainly, tolerance of tissues to low temperatures is fixed. Modifications in resistance that are known to occur in mammals generally, therefore, belong to the processes or properties that oppose tissue cooling, but cooling is exceedingly fast when a small mammal is immersed. The only exception in tissue susceptibilities is the spontaneous transformation of some mammals into hibernating ones. The rat's measures for protection all fall into the class of provisions that will delay cooling. Artificial provisions will evidently fall into the same class. Immersion in cold water furnishes more rapid cooling of a rat than any other means that has so far been tested. It yields more uniform cooling than is attained in air or other media. It provides a quick test of

tissue tolerance to cold. Further, it shows that survival temperatures of tissues are the same whether the rat be cooled slowly or rapidly. Over longer periods of time than the 2-hour and 5-hour periods that we have used, time might become a factor in survival, as was shown to be the case in guinea pigs (1). In the present experiments a period of four hours at 15° colonic temperature killed few more rats than a period of one hour; while a period of 12 hours at 16° was lethal. The percentage of delayed deaths may or may not be greater in the prolonged immersions.

Simultaneous measurements of esophageal and colonic temperatures in rats showed that during partial immersion in water of 12°C., a temperature gradient of 3 to 4°C. can be maintained between anterior and posterior portions of a rat as small as 200 gm. In that water the gradient makes the difference between death and life. Such a gradient can exist transversely in this small animal only when the temperature of the body is very high (above 30°). At low temperatures the rate of heat production is too low to support it. The lethal temperature during water immersion is surprisingly constant among individuals and with age above 26 days after birth. Moreover, no treatment has yet been found to influence the ultimate survival. Many other treatments need testing in the hope that some will prove effective.

#### SUMMARY

Median lethal temperatures were ascertained by immersing mature rats for 2 hours in waters of constant temperatures. The median lethal temperature of surrounding water was 14.8°C., its standard deviation was  $\pm 0.5^\circ$ . The colonic temperatures were ordinarily 0.1° to 0.3° higher than the water temperatures. Rates of cooling were not significant factors in the survival of rats after lethal cooling. Sometimes after incomplete rewarming, death was delayed for many hours after apparent recovery. No factor was found to modify the lethal temperature of the unanesthetized rat. Age above one month, season, body size, sex; administrations of glucose or cortin, digitalis or oxygen; deprivations of food or of water; acclimatization to cold air, all failed to modify the lethal temperature. Resistance to cold in rats appears not to be modifiable, therefore, in the tolerance of tissues, but depends solely upon delays in the cooling of essential tissues.

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# INFLUENCE OF TEMPERATURE ON THE RESPONSE OF FROGS TO X IRRADIATION

HARVEY M. PATT AND MARGUERITE N. SWIFT

*From the Biology Division, Argonne National Laboratory*

CHICAGO, ILLINOIS

**I**N A preliminary note, we reported that low temperature treatment of frogs after total-body exposure to x-rays greatly prolongs their survival (1). The present studies were initiated to determine: 1) whether this altered radiosensitivity is due to a decrease in the rate of development of radiation injury or actually reflects a more rapid and complete recovery from the effects of irradiation and 2) whether body temperature during exposure influences the radiation reaction. Since the overt response of the frog, a poikilotherm, to x-radiation appears to be rather similar in many respects to that observed in the mammal, it was thought that these studies might be of practical as well as theoretical interest.

## METHODS

Male frogs (*Rana pipiens*) weighing 20 to 30 gm. were housed in stainless steel or glass aquaria provided with platforms to enable them to remain in or out of water. The stock animals were kept in a constant temperature room (22°–23°C.) and were not fed during the experiment. Each shipment of frogs was observed for 2 to 4 weeks before irradiation and those animals obviously in poor condition were discarded. In spite of all precautions, some deaths occurred from time to time among the stock animals. To control this spontaneous mortality, nonirradiated frogs were observed along with their irradiated members in each experiment. Moreover, in each experiment the various conditions of temperature were evaluated on groups of animals received at the same time.

Frogs were irradiated in a circular cell of perforated aluminum divided into 10 radially arranged individual compartments. The exposure box was rotated slowly on an electrically driven turntable to assure equal irradiation of all the animals. The radiation factors were: 200 kv.; 15 ma.; 0.5 mm. Cu. and 3.0 mm. bakelite filters; target distance 45 cm.; dose rate 45–50 r/min., total dose 1000 to 9000 r.

For irradiation at different body temperatures, the exposure box was fitted into the inner chamber of a cylindrical constant temperature cell. Water or ice was placed in the concentric outer chamber to achieve the desired ambient temperature. Except for the lucite top of the inner chamber through which the x-ray beam was directed, the entire cell exterior was insulated with rock wool. Air temperature of the inner chamber did not vary by more than  $\pm 1^\circ\text{C}$ . from the beginning to the end of an exposure. Frogs were placed in the constant temperature exposure cell two hours before irradiation. Rectal temperature measurements made with copper-constantan thermocouples revealed that this interval was sufficient to assure body and ambient temperature equilibrium, with the former usually some few tenths of a degree centigrade above the latter. Animals which were kept at low temperatures after x-irradiation were placed in large refrigerators equipped with special thermostats.

Survival was the major criterion of radiation effect. In a pilot series, the radiation-induced changes in blood cell counts and in the histopathology of certain tissues were also compared under different conditions of temperature. Hematological data were obtained in frogs given 3000 r at 23°C. subsequently kept at 5°C. for 28 days and then returned to 23°C. Counts were made at

weekly intervals on three frogs in each experimental group. Comparison was made with appropriate controls.

### RESULTS

Toxicity data for animals maintained at different ambient temperatures after exposure to various roentgen doses delivered at 22° to 23° C. are summarized in table 1. The prolonged survival with low-temperature treatment is clearly evident even for a radiation dose (9000 *r*) which is almost nine times the completely lethal dose. Some 80 to 90 per cent of the animals kept at 5° C. for three to four months after irradiation survive a dose (3000 *r*–6000 *r*) which kills all frogs at 22° C. within two to five weeks. However, when the former are removed from the cold even after

TABLE 1. INFLUENCE OF TEMPERATURE UPON SURVIVAL OF FROGS AFTER X IRRADIATION<sup>1</sup>

DOSE, <i>r</i>	TEMPERATURE, °C.	NO. OF FROGS	PERCENTAGE SURVIVAL—WEEKS AFTER X IRRADIATION									
			1	2	3	4	5	6	7	8	13	18
1000	23	38	97	95	82	58	27	19	19	13		
1000	6	20	100	100	95	95	90	90	90	90		
3000	22	129	95	79	32	5	0					
3000	12	10	90	80	70	70	70					
3000	5	58	100	100	98	96	96	94	92	92	80 <sup>2</sup>	
6000	23	12	100	0								
6000	5	12	100	100	100	100	100	100	100	100	100	100
9000	22	12	100	0								
9000	5	12	100	100	100	100	75	42	0			

<sup>1</sup> All animals were irradiated at 23°C.    <sup>2</sup> Only 30 of the original group of 58 animals were observed at 13 weeks.

prolonged periods, death ensues. The time for 50 per cent lethality at 23° C. after varying periods of low-temperature treatment is presented in figure 1. It will be noted that ultimate toxicity is not influenced significantly by keeping frogs at 5° to 6° C. for 60 days after 1000 *r*, 75 days after 3000 *r* and 130 days after 6000 *r*. Mortality of the nonirradiated temperature controls averaged only 5 to 10 per cent over these intervals. Inspection of figure 2 reveals that the survival curve for the 1000 *r* frogs after their removal from low temperature is identical with that obtained for similarly exposed animals without cold treatment. Similar results were observed in the 3000 *r* and 6000 *r* groups.

The ambient temperature at which frogs are x-irradiated is clearly without effect on survival (fig. 2). Similar responses are obtained in the groups exposed to 1000 *r* at 1° to 3° C. and at 21° to 23° C. Combining low temperature during exposure to 1000 *r* and for the first 24 hours after exposure likewise does not influence toxicity. Percentage survival and the mean survival time are also not altered appreciably when frogs are irradiated with 3000 *r* at 5° C., 12° C., 21° C. and 32° C. (fig. 3).

When the blood counts of irradiated frogs are expressed as percentage changes from their respective temperature controls, no definite trends are discernible in red cell number. Blood platelets tend to decrease (6th day), then increase (13th and



20th days) after irradiation. These changes seem less severe in the animals kept at low temperature after exposure. However, the clearest indication of a tempera-

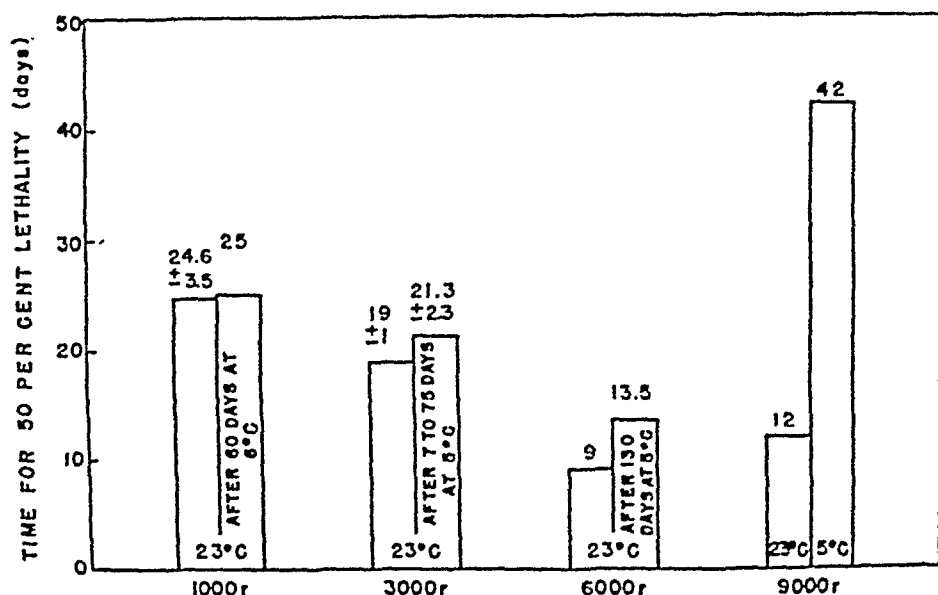


Fig. 1. EFFECT OF LOW TEMPERATURE TREATMENT upon survival of X-irradiated frogs after their removal to 23°C. (All animals irradiated at 23°C. Period of cold treatment not included in calculations of 50% survival time except for the 9000 r group in which deaths occurred at low temperature.)

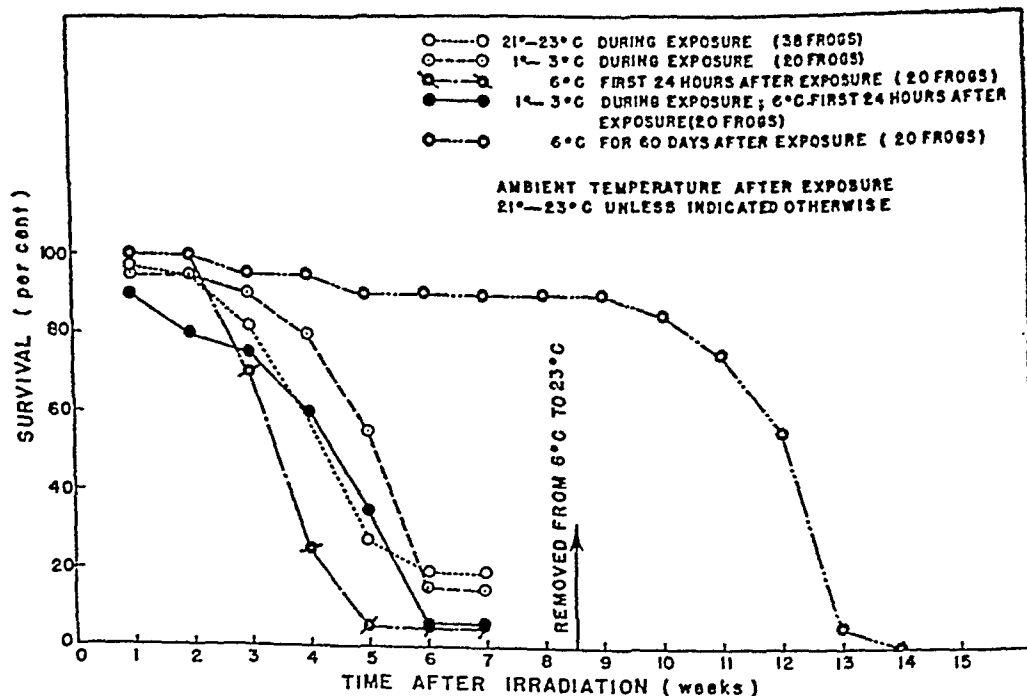


Fig. 2. EFFECT OF TEMPERATURE upon survival of X-irradiated frogs (1000 r).

ture effect is in the total white cell count, which decreases to a lesser extent in the irradiated frogs kept at low temperature. Yet, when the cold-treated animals are returned to room temperature after 28 days, their leukocyte count is depressed and reaches a low level comparable to that seen in the nonrefrigerated group (fig. 4).

Histological studies on frogs sacrificed at 2, 4, 6, 24 and 48 hours following exposure to x-rays at 23° C. reveal that cellular degeneration of lymphocytes in the spleen and intestinal submucosa is evident at 2 hours in animals kept at 23° C. and at 48 hours in those at 5° C. There is apparently no further damage after 6

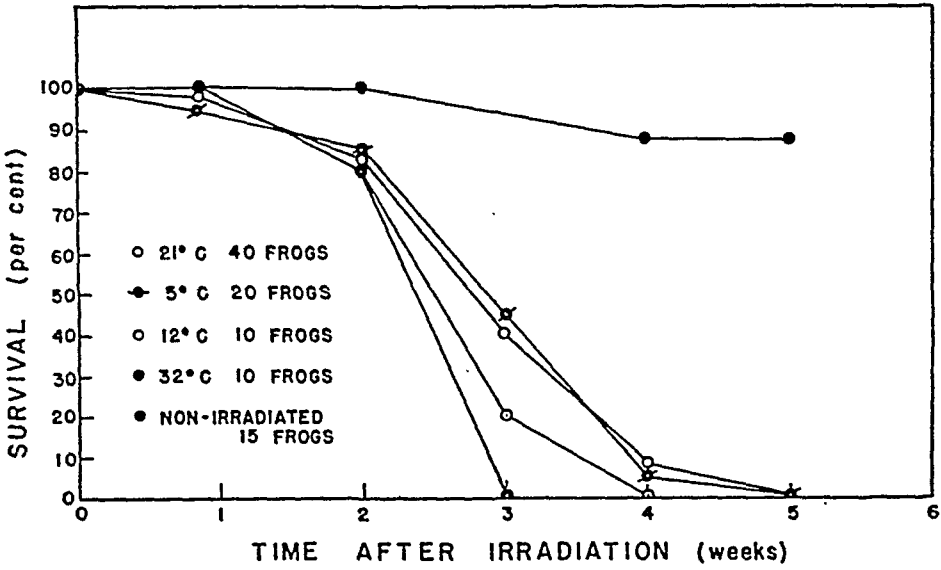


Fig. 3. EFFECT OF TEMPERATURE during x-irradiation on survival (3000 r).

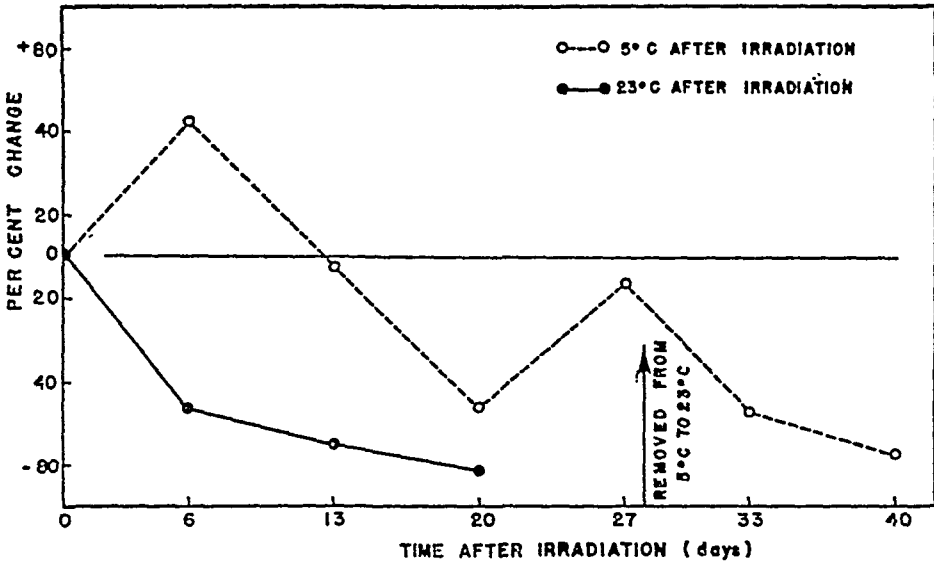


Fig. 4. INFLUENCE OF TEMPERATURE on leucocyte count of irradiated frogs (3000 r).

hours in the animals kept at room temperature, and at 24 and 48 hours there is no appreciable difference in these animals from the nonirradiated temperature controls.

#### DISCUSSION

There is evidence which suggests that the radiosensitivity of tissue may be dependent upon certain aspects of its activity. An increase in growth rate, cell division, respiratory activity, blood flow etc., induced by temperature, drugs and other

experimental manipulation, is said to enhance susceptibility to radiation under certain conditions (2-8). On the other hand, a change in cell environment can also alter the response to ionizing radiation independently of a change in the number of dividing cells and of oxygen consumption, or can even fail to influence sensitivity though growth rate and metabolic activity are greatly diminished (5, 9).

The eggs of *Ascaris* and *Drosophila* and the skin of young rats irradiated at low ambient temperature are more resistant than those at high temperature (2, 4, 6). The reverse is true for the broad bean root and for mouse tumor tissue (9, 10). Radiosensitivity of wheat seedlings and of thymic cell suspensions is not affected by low temperature (5, 11). When chick embryos and *Ascaris* eggs are maintained at low temperature after exposure, radiation injury is delayed in the former and apparently repaired in the latter (12, 13). Similar experiments with the broad bean root, *Ascaris* eggs and thymic cell suspensions, in which desiccation, an oxygen-poor environment and low temperature respectively were utilized to decrease cellular activity after irradiation with gamma and x-rays, reveal that there is no appreciable recovery under these conditions (14, 11). Many of these findings are difficult to reconcile solely on the basis of differences in the test objects and in the criteria of radiation effect employed and are perhaps indicative of the complexity of radiation reactions.

Survival of frogs is not influenced by altering their body temperature during and/or for the first 24 hours after x-irradiation. Similarly, we noted in other experiments that toxicity was unchanged when the oxygen consumption of frogs was depressed by some 50 per cent during exposure to x-rays as a result of a prior injection of potassium cyanide (15). Survival is greatly enhanced, however, as long as the frogs are kept at low temperature continuously after irradiation. Altered sensitivity in the cold is due apparently to a decrease in the rate of development of radiation damage (prolongation of the latent period) rather than to any appreciable recovery. When the animals are removed from the cold after a prolonged period, there is no change in absolute survival nor any clear difference in the time course of deaths from that observed in irradiated animals maintained at 23° C. Additional evidence in support of this interpretation is presented in the hematologic and histologic findings in the few frogs in which these determinations were made.

We may conclude from these studies in frogs that the primary reactions occurring during exposure to ionizing radiation, which initiate the changes leading to morbidity, are independent of body temperature over a wide range and are apparently physical or photochemical in nature. The changes incidental to irradiation (secondary reactions) are, however, temperature sensitive. Yet, modifying the rate of these secondary reactions by depressing body temperature for prolonged periods is without effect on the final outcome. This suggests that the mechanisms concerned with recovery may, likewise, have a high temperature coefficient.

#### SUMMARY

Toxicity is not influenced by altering the body temperature of frogs during and/or for the first 24 hours after total-body x-irradiation with 1000 r and 3000 r. Survival is greatly enhanced, however, as long as the animals are kept in the cold (5°-6° C.)

continuously after the exposure. This altered sensitivity is due apparently to a decrease in the rate of development of radiation damage (prolongation of the latent period) rather than to any appreciable recovery. When the animals are removed from the cold after periods of 60 to 130 days, there is no change in absolute survival nor any clear difference in the time course of deaths from that observed in irradiated animals maintained at 23° C. These findings indicate that the metabolic level during exposure does not influence the overall radiation reaction in frogs and that the primary process of radiation damage is not repaired in the metabolically depressed animal.

The authors wish to thank Miss Ruth Rhoades for her cooperation in the preparation and interpretation of the tissue sections.

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# AN ALTERED PROTHROMBIN PRODUCED BY DICUMAROL-TREATED RABBITS<sup>1</sup>

JOSEPH LEIN<sup>2</sup> AND PATRICIA S. LEIN

*From the Marine Biological Laboratory*

WOODS HOLE, MASSACHUSETTS

*And the Department of Zoology, Syracuse University*

SYRACUSE, NEW YORK

SCHOFIELD (1) was the first investigator to describe the hemorrhagic disease of cattle known as sweet clover disease. The work of Roderick (2, 3) indicated that the increased clotting time was primarily due to a deficiency in the prothrombin content of the blood. The causative agent of this disease was isolated by Campbell and Link (4) and the chemical studies of Stahmann *et al.* (5) showed it to be 3,3'-methylenebis(4-hydroxy-coumarin) known popularly by the trade name of Dicumarol. Further work with Dicumarol tended to confirm the hypothesis that the substance prevents the synthesis of prothrombin in the liver (6-9). The evidence for this view has been obtained by determining the clotting time of plasma from treated and untreated animals using excess protein thromboplastic agents and optimal quantities of calcium ions. The clotting time of the animals treated with Dicumarol was markedly increased and it was thought that this indicated a lack of prothrombin since the other necessary clotting factors were present in excess.

During a study of the effect of Dicumarol on the prothrombin content of dog plasma, it was noted that clotting of such plasma occurred more rapidly with a lipid thromboplastic agent than with a protein thromboplastic agent. Since it has been frequently shown that the protein thromboplastic agents are more active than the lipid ones (10, 11), this anomalous behavior was investigated.

## METHODS

### *Reagents*

*Lipid thromboplastic agent.* This agent was prepared from beef brain by the procedure of Hays and Lein (11). It consists of a mixture of phospholipids and gave no evidence of containing protein by the xanthoproteic test. The material was protected from autoxidation by shaking with a saturated solution of hydroquinone in acetone, collecting on a Büchner funnel and drying *in vacuo*. This material showed an optimal thromboplastic activity at a concentration of 3 mg/ml. of 1 per cent NaCl. An emulsion of the lipid at this concentration was prepared weekly and kept at 5°C. when not in use.

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Received for publication October 18, 1948.

<sup>1</sup> This work was done during the senior author's tenure of a Lalor Fellowship at the Marine Biological Laboratory.

<sup>2</sup> Present address: Department of Zoology, Syracuse University, Syracuse, N. Y.

*Protein thromboplastic agent.* Beef brain served as a source of this agent. The brains were stripped of their meninges and macerated in two volumes of 1 per cent NaCl. The mixture was placed at 5°C. for 20 hours and then strained through cheesecloth. The liquid was centrifuged at 2000 r.p.m. for 30 minutes and an equal volume of saturated ammonium sulfate was added to the supernatant. After 30 minutes at 5°C. a flocculent precipitate settled out and was packed by centrifugation. The tan residue was dissolved in one per cent NaCl, one part of saline to six parts of the original weight of brain tissue. This protein thromboplastic agent was stable over a period of at least one month. The small amount of ammonium sulphate present in the preparation was not removed by dialysis. Experiments on the clotting of diluted plasma by the protein agent indicated that the concentration of ammonium sulphate was too small to have an inhibitory effect under the conditions in which the protein agent was used.

*Fibrinogen.* The fibrinogen was prepared from beef blood by the method of Jacques (12). This method consists essentially of precipitating the protein three times in a 1M phosphate solution of pH 6.6 and dialyzing it to get rid of the phosphate.

*Prothrombin.* Two types of prothrombin preparations were used. One, derived from beef blood, was kindly supplied by Dr. Walter H. Seegers. It was a very highly purified prothrombin obtained by the method of Ware and Seegers (13). The preparations of prothrombin from rabbit plasma were made using the procedure of Munro and Munro (14). This method is based upon the adsorption of prothrombin on aluminum hydroxide and its elution with a 0.2M phosphate buffer of pH 8.0.

#### PROCEDURE

Experiments were carried out using rabbit plasma. Blood was drawn by cardiac puncture directly into 0.13M sodium citrate, using one part of citrate to nine parts of blood. The blood was immediately centrifuged at 2000 r.p.m. for 25 minutes and the plasma removed. Clotting times were determined in Pyrex tubes, 10 x 75 mm., using 0.6 ml. of plasma, 0.2 ml. of thromboplastic agent and 0.2 ml. of 1 per cent calcium chloride. After mixing the reagents the time in seconds when the tubes could be inverted without flow of contents was determined. This was considered to be the clotting time. These times were determined at least in duplicate and the mean values are recorded in the data. Clotting times were also determined with 1 per cent NaCl substituted for the thromboplastic agents. These mixtures clot due to some extraneous thromboplastic agent being present in the plasma. All experiments were carried out at room temperature.

#### RESULTS

*Clotting of Dicumarol plasma.* A total of 9 rabbits was used in the Dicumarol experiments. The clotting times were first determined before giving them Dicumarol. Dicumarol, 10 mg/kg., was then mixed with a small quantity of the rabbits' food, the bulk of the food being withheld until all the treated food had been consumed. The Dicumarol was given on three successive days and a blood sample was withdrawn on the fourth day. The animals showing prolonged clotting times invariably died. Autopsy revealed excessive bleeding into the pericardial cavity.

The plasma obtained from 6 of the Dicumarol-treated rabbits was divided into two portions. The clotting times were determined on one of these portions. To the other, excess purified beef prothrombin in the form of powder was added and the clotting times noted. This was done to determine if normal relationships would be restored in Dicumarol plasma by the addition of purified prothrombin. The clotting times in seconds of this series of experiments are presented in table 1.

The data of the table show that with normal plasma the protein thromboplastic agent is considerably more active than the lipid agent. In 7 of the 9 rabbits this relationship is drastically changed in the Dicumarol plasma. In 4 of these rabbits, 1, 2, 3 and 6, the Dicumarol plasma did not clot with the protein thromboplastic agent in over 11,000 seconds while the lipid agent induced clotting in an average of 404 seconds. Rabbits 5, 8 and 9 also showed markedly longer clotting times with

TABLE 1. CLOTING OF NORMAL PLASMA, DICUMAROL PLASMA AND DICUMAROL PLASMA PLUS PROTHROMBIN BY SODIUM CHLORIDE, THE LIPID THROMBOPLASTIC AGENT AND THE PROTEIN THROMBOPLASTIC AGENT

RABBIT NO.	CLOTING TIMES								
	Normal plasma			Dicumarol plasma			Dicumarol plasma plus prothrombin		
	NaCl	Lipid	Protein	NaCl	Lipid	Protein	NaCl	Lipid	Protein
1	186	70	57	1135	443	>11,000			
2	178	79	50	1620	420	>11,000	273	95	87
3	189	100	63	1425	293	>11,000	210	100	57
4	288	132	53	563	273	183			
5	223	91	63	1350	600	1,380	161	71	47
6	245	94	57	1785	460	>11,000	160	85	58
7	193	70	53	275	110	100			
8	327	110	53	1468	193	2,480	160	70	40
9 <sup>1</sup>	305	77	39 <sup>1</sup>	1560	263	3,600	180	63	38 <sup>1</sup>

<sup>1</sup> A new preparation of the protein thromboplastic agent was used in this experiment.

the protein agent as compared with the lipid agent. Rabbits 4 and 7 did not show these marked effects. Campbell *et al.* (15) showed that some rabbits are more resistant to Dicumarol than others and that the resistance is inherited in simple Mendelian fashion. It is thought that rabbits 4 and 7 may be Dicumarol-resistant rabbits. Results also indicate some increase in the clotting time of Dicumarol plasma by the lipid. These values are invariably longer than those of the normal plasma. Adding highly purified prothrombin to the Dicumarol plasma caused the clotting times to revert to the condition found in normal plasma.

It is interesting to note in the data that the 7 rabbits showing the altered clotting relationship of protein and lipid thromboplastic agents also have a longer clotting time when protein thromboplastic agent is added than when one per cent NaCl is used. This indicates that the protein agent is actually inhibiting the clotting of the plasma by the thromboplastic agent normally present in the plasma. An experiment was carried out to see if the protein would inhibit clotting of the Dicumarol plasma by the lipid agent. Clotting time was determined on Dicumarol plasma of

*rabbit 3* using in one case 0.6 ml. of plasma, 0.1 ml. one per cent NaCl, 0.1 ml. lipid agent and 0.2 ml. one per cent  $\text{CaCl}_2$ . The plasma clotted in 285 seconds. A second tube containing 0.6 ml. of plasma, 0.1 ml. protein agent, 0.1 ml. lipid agent and 0.2 ml. of one per cent  $\text{CaCl}_2$  clotted in 660 seconds. This experiment shows that the protein actually does act as an inhibitor of clotting in Dicumarol plasma.

*Physical reduction of prothrombin content.* In view of the results obtained with Dicumarol plasma, experiments were carried out in which the prothrombin content of normal plasma was reduced by physical methods. The clotting times of such plasmas obtained with the lipid and protein thromboplastic agents would indicate whether the Dicumarol results could be explained on the basis of reduction of prothrombin content of the plasma or whether some other explanation would be necessary. The prothrombin in normal plasma was reduced in concentration by two methods. In the first the prothrombin was reduced by dilution and in the second it was reduced by adsorption on aluminum hydroxide.

TABLE 2. CLOTTING OF DILUTED PLASMA BY LIPID AND PROTEIN THROMBOPLASTIC AGENTS

% OF NORMAL PLASMA	CLOTTING TIMES			
	Diluted with 1 % NaCl		Diluted with fibrinogen solution	
	Lipid	Protein	Lipid	Protein
100	61	52	41	39
50	86	58	45	41
25	105	70	54	49
12.5	130	106	99	90
6.3	220	143	135	109
3.1	315	285	276	175
1.5			435	294

Two samples of plasma were used for the dilution series. One sample was diluted using one per cent NaCl as the diluent while the other was diluted using fibrinogen solution. Clotting times were determined at the various dilutions using the diluted plasmas, thromboplastic agents and  $\text{CaCl}_2$  in proportions described in the methods section. These results are presented in table 2.

It will be seen from the data of table 2 that when normal prothrombin is decreased in concentration by dilution, clotting by the protein thromboplastic agent is always more rapid than by the lipid. Even though the clotting time of the more dilute plasmas clotted by the lipid agent were of the same order of magnitude as that of the Dicumarol plasma of table 1, clotting times using the protein agent were still shorter.

The prothrombin concentration was also reduced in normal plasma by adsorbing the prothrombin on various amounts of aluminum hydroxide cream.<sup>3</sup> This preparation has been shown by Munro and Munro (14) to adsorb prothrombin. The aluminum hydroxide cream was added to normal rabbit plasma to make volume con-

<sup>3</sup> The aluminum hydroxide cream used was Wyeth's 'Amphojel' without flavor. This was kindly supplied by Doctor Barol of Wyeth Corporation.



centrations of 1, 2, and 3 per cent. It was mixed thoroughly and allowed to stand at room temperature for 15 minutes. After this time the aluminum hydroxide was centrifuged down and the plasma used for the clotting time tests. Clotting times were determined as before using the adsorbed plasmas and the protein and lipid thromboplastic agents. Clotting times with one per cent NaCl instead of the thromboplastic agents were also determined. The experimental results are presented in table 3.

The data of table 3 indicate that if one reduced the prothrombin content of plasma by adsorption on aluminum hydroxide, the protein thromboplastic agent still shows greater activity than the lipid agent.

*Isolation of altered prothrombin from Dicumarol plasma.* The above experiments indicate that reducing the prothrombin concentration by physical methods gives

TABLE 3. CLOTTING OF ALUMINUM HYDROXIDE-TREATED PLASMA BY SODIUM CHLORIDE, THE LIPID THROMBOPLASTIC AGENT AND THE PROTEIN THROMBOPLASTIC AGENT

% Al(OH) <sub>3</sub>	CLOTTING TIMES		
	NaCl	Lipid	Protein
0	173	78	60
1	558	141	111
2	1020	190	135
3	1320	255	185

TABLE 4. CONVERSION OF PROTHROMBIN ISOLATED FROM NORMAL AND DICUMAROL PLASMA TO THROMBIN BY THE LIPID AND PROTEIN THROMBOPLASTIC AGENTS

TYPE OF PLASMA	CLOTTING TIMES		
	NaCl	Lipid	Protein
Normal.....	195	95	85
Dicumarol.....	690	443	1005

different results in relation to thromboplastic clotting than does treatment of rabbits with Dicumarol. It was thought advisable therefore to attempt an isolation of prothrombin from plasma of a rabbit treated with Dicumarol and compare it with prothrombin isolated from a normal rabbit with respect to activation by protein and lipid thromboplastic agents. Prothrombin was isolated by the technique of Munro and Munro (14) from plasma of a normal rabbit and the Dicumarol plasma of rabbit 6. These prothrombin solutions had a Kjeldahl nitrogen value of 5 and 6 mg. per cent and thus represented a purification of the order of 250 times. Experiments on the conversion of prothrombin to thrombin by lipid and protein thromboplastic agents were carried out by determining the clotting time of 0.3 ml. of fibrinogen, 0.1 ml. of prothrombin, 0.1 ml. of one per cent CaCl<sub>2</sub> and 0.1 ml. of the thromboplastic agents. Similar tubes were set up using 0.1 ml. of one per cent NaCl instead of the thromboplastic agents. Since clotting did occur in these latter tubes, it is evident that an extraneous thromboplastic agent normally present in plasma

had been carried over in the preparation of either the fibrinogen or the prothrombin or both. The clotting times in seconds of these mixtures are given in table 4.

The data of table 4 are in agreement with the results found using whole Dicumarol plasma. The prothrombin from untreated rabbits was converted to thrombin more rapidly by the protein thromboplastic agent than by the lipid. In the case of the prothrombin isolated from the Dicumarol-treated rabbit, the clotting time of the protein agent was longer than that of the control saline solution, while the lipid clotting time was considerably shorter than that of the control. Thus it appears that the thromboplastic lipid is able to convert to thrombin the purified prothrombin obtained from plasma of Dicumarol-treated rabbits while the protein agent is inactive. Furthermore, the protein agent actually inhibits clotting of these solutions by the extraneous thromboplastic agent present in the preparations. Substantially the same results were obtained with another isolation from Dicumarol plasma.

#### DISCUSSION

The fact that a lipid thromboplastic agent clots the plasma of Dicumarol-treated rabbits, whereas the normally more potent protein thromboplastic agent is ineffective, necessitates a change in concept either of thromboplastic action or of Dicumarol action. An unlikely explanation is that there are two prothrombins in the blood, one activated by the protein thromboplastic agent and the other by the lipid thromboplastic agent. With this hypothesis one would assume that the Dicumarol affects primarily the synthesis of the latter prothrombin. This is thought unlikely, since adding highly purified prothrombin to Dicumarol plasma will not only make it clottable by a protein clotting agent but, also, reduces the somewhat prolonged clotting time of the lipid thromboplastic agent to its normal value. This indicates that both the lipid and the protein thromboplastic agents can react with the same prothrombin.

It is believed that the results can best be explained by assuming that Dicumarol does not prevent the formation of prothrombin by the liver but changes the specificity of prothrombin formed by the liver in such a way that it is not activated by protein thromboplastic agents but is activated, though with decreased efficiency, by lipid thromboplastic agents. Thus, consider the blood of *rabbit 6*. Before Dicumarol, its clotting time with a protein thromboplastic agent was 57 seconds. After Dicumarol treatment it was greater than 11,000 seconds. From these values and the usual concept of Dicumarol action, one would expect that the plasma was practically devoid of prothrombin. Yet, the plasma that did not clot in 11,000 seconds with a protein thromboplastic agent did clot with a lipid thromboplastic agent in 460 seconds. Furthermore, the 'prothrombin' in the Dicumarol plasma was purified and experiments showed it to be acted on by the lipid agent but not by the protein agent. We think that this is strong evidence for the presence of an altered prothrombin in Dicumarol plasma, especially since experiments in which the prothrombin content of normal plasma is decreased by either dilution or adsorption on aluminum hydroxide show that the protein thromboplastic agent is always more active than the lipid.

The somewhat prolonged clotting time of Dicumarol plasma with the lipid throm-

boplastic agent could indicate either that the altered prothrombin is present in a lower concentration than normal plasma prothrombin or that the efficiency of conversion of the altered prothrombin by the lipid is decreased. The possibility that both of these contribute to the prolonged clotting time also exists. While no rigorous argument could be developed from the data favoring any of these choices, it is believed more probable that the lipid agent acts on the altered prothrombin with less efficiency. The very fact that the prothrombin has a changed structure makes it unlikely that it will react with the lipid thromboplastic agent with the same reaction velocity constants as does native prothrombin.

The results further indicate that the protein thromboplastic agent actually inhibits the conversion of prothrombin to thrombin by the lipid thromboplastic agent. This behavior readily explains the results of the workers who used crude thromboplastic mixtures containing both protein and lipid thromboplastic substances. Such a mixture is the thromboplastin prepared by Quick's method (16) and used extensively by workers to determine clotting times of Dicumarol plasma. The protein thromboplastic agent in these preparations probably prevents the lipid thromboplastic agent from exerting its effects. Consequently, measurements using such thromboplastic preparations would be the same as those obtained using protein thromboplastic agents.

The clotting of recalcified Dicumarol plasma, when one per cent NaCl is used instead of a thromboplastic agent, indicates that the extraneous thromboplastic substance present in the rabbit plasma has the characteristics of the lipid thromboplastic agent. Its efficiency of action is decreased in the case of Dicumarol plasma and its action is inhibited by the protein thromboplastic agent. This view receives further support from the experiments studying the conversion to thrombin of prothrombin purified from Dicumarol plasma. It was shown that considerable extraneous thromboplastic substance is carried over in the preparation of either the prothrombin or the fibrinogen and this substance behaves in much the same fashion as the lipid thromboplastic agent.

#### SUMMARY

Experiments were carried out on the clotting of plasmas of untreated rabbits and rabbits treated with Dicumarol using lipid and protein thromboplastic agents. While with normal rabbit plasma the protein thromboplastic agent is more active than the lipid agent, the relationship is reversed after treatment with Dicumarol. In one case Dicumarol plasma which did not clot in three hours with the protein agent clotted within five minutes with the lipid agent.

Since reduction of prothrombin concentration by dilution or by adsorption on aluminum hydroxide does not reverse the relative thromboplastic activities of the lipid and protein agents, it is assumed that Dicumarol treatment does not prevent the synthesis of prothrombin by the liver but causes the synthesis of an altered prothrombin. This altered prothrombin is not converted to thrombin by the action of a protein thromboplastic agent but is converted by the action of a lipid agent.

It has been shown that the protein thromboplastic agent inhibits the clotting of Dicumarol plasma by the lipid thromboplastic agent. This fact is thought to ex-

plain the results of prothrombin time determinations on Dicumarol plasma using a thromboplastic agent containing both the lipid and protein agents. The thromboplastic substance normally present in rabbit plasma has characteristics of the lipid thromboplastic agent.

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# EFFECT OF THYROXINE AND THIOURACIL ON THE RATE OF PHOSPHOLIPID TURNOVER IN THE LIVER OF THE RAT<sup>1</sup>

EUNICE V. FLOCK, JESSE L. BOLLMAN AND JOSEPH BERKSON

*From the Division of Experimental Medicine, Mayo Foundation, and the Division of Biometry and Medical Statistics, Mayo Clinic*

ROCHESTER, MINNESOTA

CALCULATIONS based on the exchange of  $P^{32}$  from the inorganic phosphate to the phospholipids of the liver indicate that approximately 5 per cent of these phospholipids are renewed each hour in the normal adult rat (1). Very large variations of the amount of protein, carbohydrate or fat in the diet of the rat may produce alterations of the relative size of the liver and the concentration of phospholipids of the liver. The rate of phospholipid formation, however, under these circumstances was found to be essentially that of normal rats (2). A marked increase has been found in the rapidly regenerating liver after partial hepatectomy so that the amount of phospholipid formed by the remaining portion of the liver approximates that formed by the entire liver of a normal rat (3). An increased turnover rate, as indicated by an increased amount of administered  $P^{32}$  incorporated in the hepatic phospholipid, has been found after the administration of choline (4), betaine, methionine (5), cystine and cysteine (6) and a decrease has been found after administration of cholesterol (7).

The purpose of the experiments reported here was to determine the changes in the distribution of administered  $P^{32}$  in the inorganic phosphate and the phospholipids of the plasma and liver in rats after the administration of thyroxine or thiouracil. Calculations made from these data indicate that the rate of turnover of the phospholipids of the liver is increased by the administration of thyroxine and decreased by the administration of thiouracil.

## EXPERIMENTAL METHODS

Adult male white rats weighing approximately 200 gm. maintained on the stock commercial diet of Friskies were fasted for 20 hours prior to the administration of  $P^{32}$ . One group of rats had previously received two daily subcutaneous injections of 0.1 mg. of thyroxine/100 gm. of body weight and a second group had received 0.1 per cent of thiouracil in the drinking water for periods of from 10 to 56 days. The basal metabolic rate of the rats receiving thyroxine was increased and sections of the thyroids, taken at the time of necropsy, showed the alterations expected after the administration of thiouracil. Twelve microcuries of  $P^{32}$  from the cyclotron at the Massachusetts Institute of Technology were injected intravenously, as dibasic sodium phosphate containing less than 0.01 mg. of phosphorus, at intervals of one-twelfth, one-fourth, one, two and four hours prior to removal of blood and liver, with the rats under pentobarbital sodium anesthesia. The inorganic phosphate and phospholipid of plasma and liver were extracted and the concentration and radioactivity of each were determined by methods previously described (1).

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Received for publication October 4, 1948.

<sup>1</sup> Read at the meeting of the American Chemical Society, Chicago, Ill., September 9-13, 1946.

## RESULTS

In both the thyroxine-treated and thiouracil-treated rats the weight of the liver and its water, total lipid, phospholipid and inorganic phosphate content were well within the range for normal rats fasted for the same interval. The plasma from the thyroxine-treated rats contained definitely increased amounts of inorganic phosphate, but normal amounts of phospholipid. The plasma from the thiouracil-treated rats

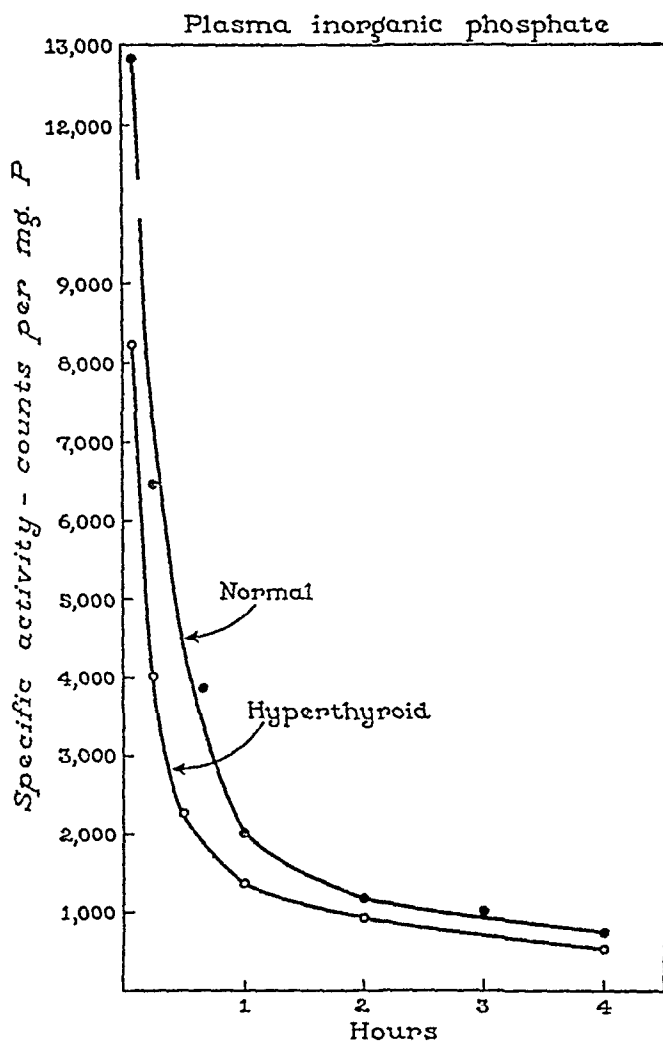


Fig. 1. CURVE OF MEAN VALUES found in normal and thyroxine-treated rats for specific activity of plasma inorganic phosphate after a single injection of phosphate  $P^{32}$ .

contained slightly less than normal amounts of inorganic phosphate and slightly more than normal amounts of phospholipid.

Because of the greater amount of inorganic phosphate in the plasma of the hyperthyroid rats, the injection of the same amount of  $P^{32}$  produced definitely lower average specific activity of the plasma inorganic phosphate than in normal rats (fig. 1), which in turn produced lower specific activity of the inorganic phosphate of the liver. The opposite was true for the thiouracil-treated rats, in which the specific activity of the inorganic phosphate of the plasma and of the liver was higher than in normal rats.

The specific activity of the phospholipids of the livers of the thyroxine-treated rats was almost identical with that of normal rats at each of the time intervals studied. The radioactivity of the phospholipid was acquired from the inorganic phosphate, which had less activity than that of the normal rats, so that more phospholipid must have been formed in the hyperthyroid rats. The relative activity, which has often been used as an indication of the rate of turnover, was 47 per cent in the hyperthyroid rats as compared with 31 per cent in the normal rats and with 22 per cent in the hypothyroid rats four hours after the administration of  $P^{32}$ . The specific activity of the phospholipids of the liver of the hypothyroid rats was definitely less than that of normal rats taken at the same time interval after administration of  $P^{32}$ .

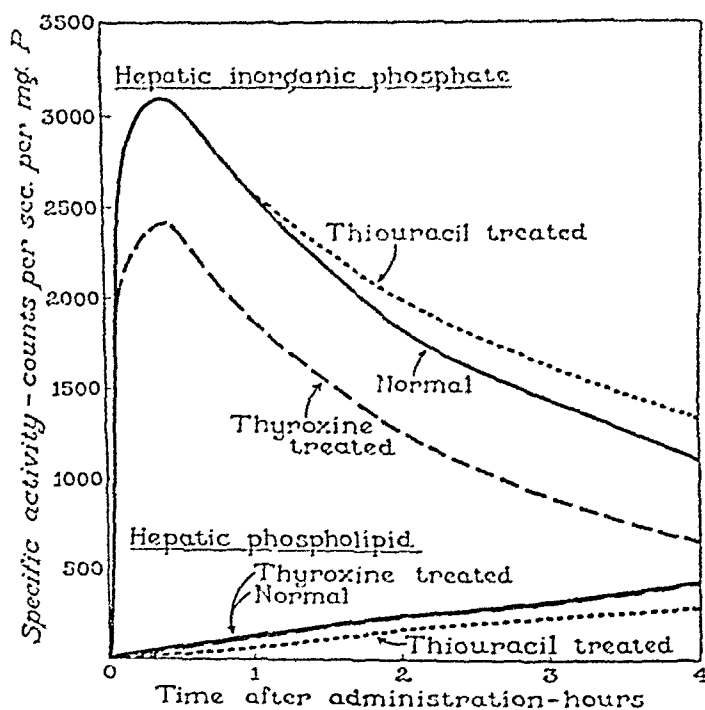


Fig. 2. CURVE OF MEAN VALUES of specific activities of hepatic inorganic phosphate and of hepatic phospholipid found after a single intravenous injection of phosphate  $P^{32}$ .

For the calculation of the rate of turnover of the phospholipids of the liver we have used the method of Zilversmit, Entenman and Fishler (8), who demonstrated that the turnover time of substance  $B$ , which is formed from the precursor  $A$ , is the quotient of the difference of the areas of the specific activity time curves of the two substances divided by the change of specific activity of  $B$  during the time interval. We have used the specific activity of the liver inorganic phosphate as that of the precursor of the liver phospholipid. The proportion of the liver phospholipids, which is renewed each hour ( $R$ ) is the quotient of the change of specific activity of the phospholipid per hour ( $d\text{ pl}$ ) divided by the difference of the mean specific activity of the liver inorganic phosphate ( $m\text{ i}$ ) and the mean specific activity of the liver phospholipid ( $m\text{ pl}$ ).  $R = (d\text{ pl})/m\text{ i} - m\text{ pl}$ . The mean specific activity for the inorganic phosphate and phospholipid phosphorus, which is required for the calculation of  $R$ , was obtained by evaluating the respective areas under the time curves (fig. 2) and

relating this to the time interval considered. The chief numbers of interest are summarized in table 1. The corresponding activity-time curves were obtained by graphic smoothing of the observed specific activities in order to depict the course of changes of these values. The numbers pertinent to the calculation of  $R$  are to be found in rows 12 through 16 of the table. The rate of change of specific activity of hepatic phospholipid per hour (row 15) is obtained by dividing the specific activity at time  $t$  (row 10) by the time (row 1). The mean specific activities of the hepatic inorganic  $P$  and phospholipid  $P$  (rows 12 and 13) were obtained from the area under the time activity curves and their differences are given in row 14. The value for  $R$

TABLE 1

	THYROXINE-TREATED RATS				THIOURACIL-TREATED RATS		
	0.5	1	2	4	1	2	4
1. Hrs. after administration, $t$ .....	6	10	12	14	6	23	38
2. Rats.....	217 $\pm$ 6	177 $\pm$ 5	180 $\pm$ 3	176 $\pm$ 2	204 $\pm$ 4	223 $\pm$ 4	210 $\pm$ 2
3. Body weight, gm.....	217 $\pm$ 6	177 $\pm$ 5	180 $\pm$ 3	176 $\pm$ 2	204 $\pm$ 4	223 $\pm$ 4	210 $\pm$ 2
4. Liver wt., % of body wt.....	3.4 $\pm$ 0.1	4.5 $\pm$ 0.3	3.7 $\pm$ 0.1	3.7 $\pm$ 0.1	3.8 $\pm$ 0.1	3.6 $\pm$ 0.2	4.0 $\pm$ 0.1
Mg. P/100 gm. (items 5 to 7)							
5. Plasma inorg. P.....	7.3 $\pm$ 0.3	9.2 $\pm$ 0.4	10.2 $\pm$ 0.4	9.4 $\pm$ 0.3	6.7 $\pm$ 0.1	5.9 $\pm$ 0.2	6.0 $\pm$ 0.2
6. Liver inorg. P.....	27.6 $\pm$ 0.6	30.4 $\pm$ 0.9	28.1 $\pm$ 0.8	31.6 $\pm$ 1.0	32.2 $\pm$ 0.3	29.8 $\pm$ 0.5	30.4 $\pm$ 0.5
7. Liver lipid P.....	129 $\pm$ 1	121 $\pm$ 4	118 $\pm$ 1	125 $\pm$ 2	126 $\pm$ 3	128 $\pm$ 2	131 $\pm$ 2
Specific activity at time $t$ (items 8 to 10)							
8. Plasma							
Inorg. P.....	2,227 $\pm$ 120	1,372 $\pm$ 38	929 $\pm$ 66	509 $\pm$ 32	2,665 $\pm$ 93	1,440 $\pm$ 55	843 $\pm$ 23
Lipid P.....		23 $\pm$ 3	164 $\pm$ 14	283 $\pm$ 7	23 $\pm$ 3	51 $\pm$ 5	185 $\pm$ 10
9. Liver inorg. P.....	2,364 $\pm$ 162	1,879 $\pm$ 61	1,267 $\pm$ 72	681 $\pm$ 46	2,585 $\pm$ 93	1,985 $\pm$ 59	1,339 $\pm$ 30
10. Liver lipid P.....	64 $\pm$ 9	110 $\pm$ 11	257 $\pm$ 14	320 $\pm$ 21	72 $\pm$ 3	179 $\pm$ 14	284 $\pm$ 9
11. Relative activity, liver lipid P: inorg. P.....	0.028	0.058	0.208	0.47	0.028	0.088	0.223
Mean sp. act. to time $t$ (items 12 to 14)							
12. Liver inorg. P.....	1,061	2,038	1,800	1,375	2,641	2,463	2,062
13. Liver lipid P.....	30	62	123	206	36	80	154
14. Difference.....	1,931	1,976	1,677	1,169	2,605	2,383	1,908
15. Change of specific activity of lipid P/hr....	128	110	128	80	72	89	71
16. $R$ .....	0.066	0.056	0.076	0.069	0.028	0.037	0.037
17. New phospholipid P in mg/hr/100 gm. of rat	0.29	0.30	0.33	0.32	0.13	0.17	0.19

is given in row 16. The amount of phospholipid  $P$  newly formed each hour calculated for each 100 gm. of rat ( $R \times$  mg. phospholipid  $P$ /100 gm. liver  $\cdot$  liver weight/body weight) is given in row 17. This is an average of 0.31 mg. for the 42 thyroxine-treated rats and 0.18 mg. average for 67 thiouracil-treated rats, which differs by  $\pm 29$  per cent and  $-25$  per cent respectively from the average of 0.24 found for 45 normal adult rats (1) at similar periods after administration of  $P^{32}$ .

We have made similar calculations from the data, obtained one hour after administration of  $P^{32}$ , from 8 young normal rats weighing 75 gm. each. These showed a turnover rate of liver phospholipids of 0.054 and because the liver is relatively larger in the young rats than in older rats 0.33 mg. of phospholipids was being formed for each 100 gm. of rat each hour. Twelve other young rats weighing 123



gm. taken four hours after administration of  $P^{32}$  gave data from which 0.058 for  $R$  and 0.33 mg. for the amount of new phospholipid  $P$  formed each hour/100 gm. of rat were calculated. For these two groups of young animals the rate of phospholipid turnover in the liver was approximately that of normal adult rat liver, but was greater on the basis of total body weight.

On the assumption that the new phospholipid entering the plasma has the same specific activity as the average specific activity of the hepatic phospholipids, we have attempted to calculate the rate of turnover of the plasma phospholipids. The specific activity of the liver phospholipid was considered to be that of the precursor of the plasma phospholipids and calculations were made from the smoothed curves of the specific activity-time relations. There was considerable variation in the data from individual rats at the same time periods. The turnover rate, that is, the proportion of phospholipids replaced in the plasma each hour, was calculated as 0.79 from average data from 38 adult normal rats taken one, two or four hours after administration of  $P^{32}$ . Similar data from 36 thyroxine-treated rats gave 0.97 or an increase of 23 per cent over the rate found for normal rats. Sixty-seven adult thiouracil-treated rats gave 0.63 or a decrease of 20 per cent from that of the normal rats.

#### COMMENT

That the thyroid hormone may cause changes in the distribution of such inorganic constituents of the body as calcium and phosphorus was shown by Aub *et al.* (9), when they found that the hormone causes a marked increase of the excretion of both phosphorus and calcium. Aoike (10) found an increased concentration of inorganic phosphate in the blood of the rabbit associated with the increased excretion of phosphorus. Logan, Christensen and Kirklin (11) found an increased excretion of calcium without significant changes in phosphorus in the dog. Robertson (12) found decreased concentration of both serum calcium and phosphorus in active untreated thyrotoxicosis in human beings. In our hyperthyroid rats there was an increased concentration of inorganic phosphate in the plasma. A similar finding has been reported by Greenberg, Fraenkel-Conrat and Glendening (13). The more rapid disappearance of injected radioactive inorganic phosphate in the hyperthyroid rats than in normal ones is in part explained by the greater dilution which occurs in plasma containing an increased concentration of inorganic phosphate. Also the increased circulation time and increased rate of excretion of phosphorus found in hyperthyroid animals are undoubtedly contributory factors. Of particular interest in this connection is the recent observation of Greenberg, Fraenkel-Conrat and Glendening (13), of a large increase in the rate of penetration of inorganic phosphate into the muscle of hyperthyroid animals.

There is definitely a relationship between the rate of turnover of hepatic phospholipid and the rate of basal metabolism, since both rates can be elevated or depressed by the administration of thyroxine or thiouracil. This may be related to the oxygen consumption in the liver, since Taurog, Chaikoff and Perlman (14) have shown that aerobic conditions are essential for the synthesis of phospholipid from radioactive inorganic phosphate in surviving liver slices.

The validity of the assumption, made for the purpose of calculating the turn-

over rate of hepatic phospholipids, that the specific activity of the hepatic inorganic phosphates is similar to that of the immediate precursor of the hepatic phospholipids is further enhanced by these studies. Previous studies indicated that this method of calculation gave constant results when calculated from entirely different curves obtained after single injections of  $P^{32}$  or after continuous administration of  $P^{32}$  and also from 10 different intervals on those curves. The rate of phospholipid turnover in the liver of thyroxine-treated animals was found to be greater than normal when calculated at four different periods after administration of  $P^{32}$  and to be reduced consistently in thiouracil-treated animals for the three different periods used.

The specific activity of the liver phospholipids of the thyroxine-treated animals was almost identical with that of untreated animals at similar time periods after the administration of the same amount of  $P^{32}$ -labeled inorganic phosphate. If the rate of turnover of phospholipid were estimated from the percentage of the administered  $P^{32}$  which appeared in the phospholipids, no change from normal would be noted. It is obvious that any condition, as in this case an elevation of the inorganic phosphate content of the plasma, which alters the specific activity of the precursor would correspondingly alter the specific activity of any compound formed from that precursor. In the thiouracil-treated animals the specific activity of both the inorganic phosphate and the phospholipid differed from that found in normal animals; less  $P^{32}$  was incorporated in the phospholipids of the liver from inorganic phosphate of higher specific activity. The rate of turnover of phospholipids was therefore less than it would be judged to be from the percentage of the administered  $P^{32}$  which appeared in the phospholipids.

The calculation of the turnover rate of plasma phospholipids, based on the assumption that the average specific activity of the phospholipids of the liver is the same as that of the newly formed phospholipids entering the plasma, is of course open to question. The specific activity of the newly formed phospholipids of the blood may have been very different from that of the average of the liver phospholipids. However, the calculated rate of turnover did not vary greatly at different periods of these experiments when the changing values would have indicated changing rates if any appreciable disparity existed. In view of this consistency, the finding of an increased rate after administration of thyroxine and a decreased rate after administration of thiouracil may provisionally be accepted. The calculated rate of phospholipid turnover in the normal rat of 0.79 should be further checked by calculations made after continuous injection of  $P^{32}$  and also by the rate of disappearance of labeled phospholipid from the plasma (15).

#### SUMMARY

Data concerning the concentration and specific activity of the liver and plasma in organic phosphates and phospholipids were obtained from adult rats at intervals after the injection of dibasic sodium phosphate  $P^{32}$ . Because their specified activities have a constant relationship over a wide variety of experimental conditions, the specific activity of the liver inorganic phosphates appears to be identical with that of the immediate phosphate precursor of the phospholipids of the liver. Calculations based on this assumption indicate that the rate of phospholipid turnover in the liver

is increased in rats which have received thyroxine and decreased in rats which have been treated with thiouracil. The rate of turnover of the phospholipids of the plasma also appears to be greater than normal in thyroxine-treated rats and less than normal in those receiving thiouracil. The rate of phospholipid turnover in the liver of young rats is essentially the same as that of mature rats but, since the relative size of the liver is greater in young rats, more phospholipid is formed in proportion to body weight than in the adult.

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# EFFECT OF SUPRATHRESHOLD CHANGES IN BRIGHTNESS ON FORM PERCEPTION

FRANK N. LOW

*From the Department of Anatomy, The Johns Hopkins University  
School of Medicine<sup>1</sup>*

BALTIMORE, MARYLAND

THE detrimental effect of diminishing brightness on seeing during the post-sunset hours has been generally recognized although not subjected to systematic study. A subjective vagueness occurs during dusk although the general level of illumination is suprathreshold. Investigation of dark adaptation (1) has dealt almost exclusively with the measurement of the absolute threshold for the perception of light, the suprathreshold brightness range not having been studied. The investigation here reported was designed to test the effect of suprathreshold brightness changes on form perception.

Variables of experimental procedure were introduced in an effort to reproduce the essential conditions under which poor dusk seeing occurs. The approach was based on the supposition that the anticipated changes, although occurring at suprathreshold levels, were of primarily adaptive nature. Since adaptive processes have been recognized to be impaired after exposure to intense brightnesses (2, 3), a regimen of preadaptation to bright light was introduced. To simulate field conditions, where high contrasts are seldom encountered, a set of low contrast test objects was used in certain of the experiments. The fact that poor dusk seeing is accompanied by poor confidence in having seen correctly suggested the inclusion of a measure of the subject's appraisal of his own seeing ability for comparison with his measured physiological ability.

## APPARATUS

*Perimeter and test objects.* Tests were made in a white cloth testing booth on a 25-cm. perimeter, on which the test objects were illuminated by a 60-watt Mazda daylight bulb. A voltage regulator (Sorensen and Co. Model 150) designed to keep the output voltage at 115 with regulation accuracy of 0.5 per cent in a recovery time of six cycles ( $\frac{1}{16}$  sec.) on an input voltage of 95 to 125 volts was operated in the power line in all experiments. The brightness of the test object was regulated by a rheostat and measured with a Macbeth Illuminometer at arbitrarily chosen resistances. During experimentation the brightness changes were controlled by setting the rheostat at the measured resistances. An arrangement of white hand-operated blind, test object with surrounding white field 74 mm. square, and white background reflector presented to the subject's visual field a 60° circular patch of uniform brightness, which did not measurably change in brightness as the test object was alternately presented and obscured. When the blind was turned for presentation the test object was revealed in the center of the 60° field. The test objects were Landolt circles. They were photo-

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Received for publication October 18, 1948.

<sup>1</sup> The work described in this paper was done under a contract between the Office of Naval Research and Johns Hopkins University (Contract N6onr-243, Task Order III, Johns Hopkins University).

graphically printed on white background. Notation of their sizes was indicated by the mm. measure of the break. The contrast of the test object against the white background is conventionally expressed as a percentage based on relative brightnesses. The test object could be turned about its own center so that the break in the circle pointed in one of four directions; up, down, right or left.

*Adaptation booth.* A booth 4 feet X 4 feet X 8 feet was constructed for the purpose of light adapting the subjects. The inside walls were coated with magnesium carbonate. The booth was illuminated by 16 40-watt daylight fluorescent bulbs. The brightness of the front and side walls at sitting height was approximately  $+2.7$  log millilamberts, at standing height  $+2.8$  log millilamberts. During light adaptation the subjects sat in the booth, the sitting height brightness level being used in this report.

### TECHNIQUE

The test objects were presented to the subjects for positional identification, the subjects' responses being an indication of the position of the break in the circle. When the subject answered the blind was returned to the obscuring position and the test object repositioned for representation. Presentations were made in sequences of 20 each, there being in each sequence five presentations of each of the 4 possible positions. The order of positional presentation was unknown to the subject and was determined by chance, the operator working from a prepared sheet. The subjects responded promptly. Presentations were made unhurriedly at the rate of one sequence of 20 in one minute and 15 seconds. The actual time of each stimulus exposure was somewhat less than 2 seconds. When positional identification was difficult by reason of small test object size or low brightness there was a tendency for the subject's responses to lag, but forced guesses were the requirement. Even under these conditions the prescribed speed of presentation was maintained without difficulty. Special operations such as time notation etc. between consecutive sequences were found to require an interval of about 15 seconds which was adopted for routine use. When change of brightness was required the rheostat was manipulated immediately before the first presentation in the new sequence. With the test object already positioned the brightness was changed and the operator's hand then turned the blind to make the first presentation. The manipulation of the rheostat required about one second, the first presentation following in less than one second. When the experiment required a change of test object size before continuing, a longer interval than 15 seconds was found expedient. A half minute was usually allotted but this varied somewhat.

The subject was required to state his confidence in having given the positional identification correctly. After each group of 4 presentations (every 15 sec.) the subject was asked, "How many?" His answer, from 0 to 4, covered the foregoing 4 presentations and gave the number of responses which he was certain he had gotten right.

A single sequence, comprising 20 presentations, was adopted as the unit of scoring. The responses to stimulus presentation are a measure of physiological efficiency and are referred to in this report as the 'performance'. The number of correct answers are totaled for each sequence and are converted to a percentage by multiplying by five. In this form the percentile performance scores are not a direct measure of the subject's ability since there is 1 chance in 4 that any response might have been correct by chance. Therefore the percentile performance scores have been corrected as

follows;  $P_{(cor)} = (P_{(uncor)} - 25)/75 \times 100$  where  $P_{(cor)}$  represents the corrected performance score and  $P_{(uncor)}$  the uncorrected performance score. The rationale of this correction is illustrated by the following example. Suppose that a subject's true frequency of seeing is known to be 60 per cent. Then the remaining 40 per cent of presentations will have one answer in four correct by chance or 10 per cent of the total. The uncorrected performance score will be  $60 + 10 = 70$  per cent, which corrects by the above formula to the true value. The responses covering certainty of having seen correctly are a measure of the subject's conscious sensory experience and are referred to in this report as the 'confidence.' The confidence scores have been similarly converted to percentages for each sequence, but they need no correction because they possess no element of chance. After the performance scores have been corrected they are directly comparable to the confidence scores. In this report all scores are reported as percentage frequency of seeing, usually for a sequence of 20 presentations.

#### PROCEDURE

Areas far removed from the line of central vision are best suited to investigations involving brightness changes for the significant reason that photopic and scotopic acuity are nearly the same  $30^\circ$  or more peripherally (4, 5). The writer's previous measures of photopic and scotopic form acuity at  $30^\circ$  and  $60^\circ$  peripherally have shown them to be related as 7 is to 5, the former being the stronger (6). Therefore, in the whole range of possible brightnesses the form acuity ought to vary only slightly if at all for the different levels, providing the eye has had time to adapt to the new brightness. This circumstance should facilitate the interpretation of any changes observed, since it has eliminated the possibility of there being greatly different acuities for the different brightnesses used.

Preliminary experiments were performed during the development of the technique. Since the number of subjects was small these experiments are not reported in detail. The results indicated that a one-half log unit sudden diminution of brightness does not measurably depress peripheral form perception at  $30^\circ$  and  $45^\circ$  peripherally at brightnesses from  $+0.8$  to  $-1.6$  log m.L. At lesser brightnesses both confidence and performance are depressed although the absolute threshold is not passed. Differential threshold curves based on different-sized test objects are sigmoid and asymptote at 0 per cent and 100 per cent after proper correction of the performance scores.

It was considered to be of interest to demonstrate changes in form perception following suprathreshold brightness diminution at as high a level as practical to emphasize the occurrence of the phenomenon at the higher levels of brightness. A series of 12 experiments, described in table 1, was performed on a group of 11 subjects. Among these experiments are two groups of three experiments each, the first group being performed with low contrast (27%) test objects  $45^\circ$  peripherally (*expts. 1, 2, 9*) and the second with high contrast (97%) test objects  $30^\circ$  peripherally (*expts. 3, 4, 10*). In these experiments the effect of a suprathreshold brightness diminution of 2.8 log units was tested with identical technique under the three following conditions of adaptation, *a*) no preadaptation, *b*) preadaptation to  $+2.7$  log m.L. for  $1\frac{3}{4}$  hours and *c*) preadaptation to  $+2.7$  log m.L. for  $1\frac{3}{4}$  hours with pupils dilated by 5 per cent euphthalmine 15 minutes before the preadaptation began. The largest test object

was used first, the subjects receiving one series of 20 presentations at  $+1.2$  log mL., then a second sequence at  $-1.6$  log mL. and finally a third sequence at the original brightness. Then after an interval of about one-half minute the procedure was repeated with a smaller test object and finally with the smallest.

*Experiments 5 and 6* with 27 per cent contrast and 7 and 8 with 97 per cent contrast provided differential threshold curves for peripheral form perception in the fully adapted eye at the two levels of brightness used in the experiments above,  $+1.2$  and  $-1.6$  log mL. At the higher level no preadaptation was considered necessary, but in *experiments 6 and 8* which were conducted at  $-1.6$  mL. the subjects wore Navy dark adaptor goggles for one-half hour previous to the test and were not exposed to greater brightnesses in the interim. Test objects were presented for one sequence each in

TABLE 1. DATA FOR SERIES OF EXPERIMENTS

EXPT. NO.	ANGLE DEV.	TEST OBJECT SIZE	TEST OBJECT CONTRAST	TEST OBJECT BRIGHTNESS IN LOG ML	PREPARATION OF SUBJECTS
1	45°	4, 3, 2	27%	$+1.2$ and $-1.6$	None
2	45°	4, 3, 2	27%	$+1.2$ and $-1.6$	1½ hrs. at $+2.7$ log mL.
3	30°	2, 1, ½	97%	$+1.2$ and $-1.6$	None
4	30°	2, 1, ½	97%	$+1.2$ and $-1.6$	1½ hrs. at $+2.7$ log mL.
5	45°	6, 5, 4, 3, 2	27%	$+1.2$	None
6	45°	6, 5, 4, 3, 2	27%	$-1.6$	Dark adapted ½ hr. with Navy goggles
7	30°	2½, 2, 1½, 1, ½, ¼	97%	$+1.2$	None
8	30°	2½, 2, 1½, 1, ½, ¼	97%	$-1.6$	Dark adapted ½ hr. with Navy goggles
9	45°	4, 3, 2	27%	$+1.2$ and $-1.6$	Pupils dilated: 1¼ hrs. at $+2.7$ log mL.
10	30°	2, 1, ½	97%	$+1.2$ and $-1.6$	Pupils dilated: 1¼ hrs. at $+2.7$ log mL.
11	30°	2	97%	$+1.2$ and $-1.6$	None
12	30°	2	97%	$+1.2$ and $-1.6$	Pupils dilated: 1¼ hrs. at $+2.7$ log mL.

order of descending size. The test object contrast and angular deviations were the same as in the remainder of the experiments.

*Experiments 11 and 12*, utilizing the same brightness diminution as other experiments, were designed to measure the duration of the observed perceptual changes. A single high contrast (97%) test object, size 2, was used 30° peripherally. The subject received one sequence at  $+1.2$  log mL. followed by 10 sequences at  $-1.6$  log mL., after which the original brightness was restored for one sequence. The subjects were not preadapted in *experiment 11*, but in *experiment 12* were light adapted at  $+2.7$  log mL. for 1¼ hours with dilated pupils.

The group of 11 subjects who took the above 12 tests, with one exception (the writer), were students at The Johns Hopkins University. No attempt was made to select them for any reason other than convenience of schedule. Each subject received an examination by a practicing ophthalmologist. No ocular pathology other than mild routine optical defects was discovered.

## RESULTS

Figure 1 illustrates the effect of altered brightness on the form perception of low contrast (27%) test objects presented  $45^\circ$  peripherally on the horizontal meridian. The solid cross bars represent the mean corrected performance scores in 11 subjects

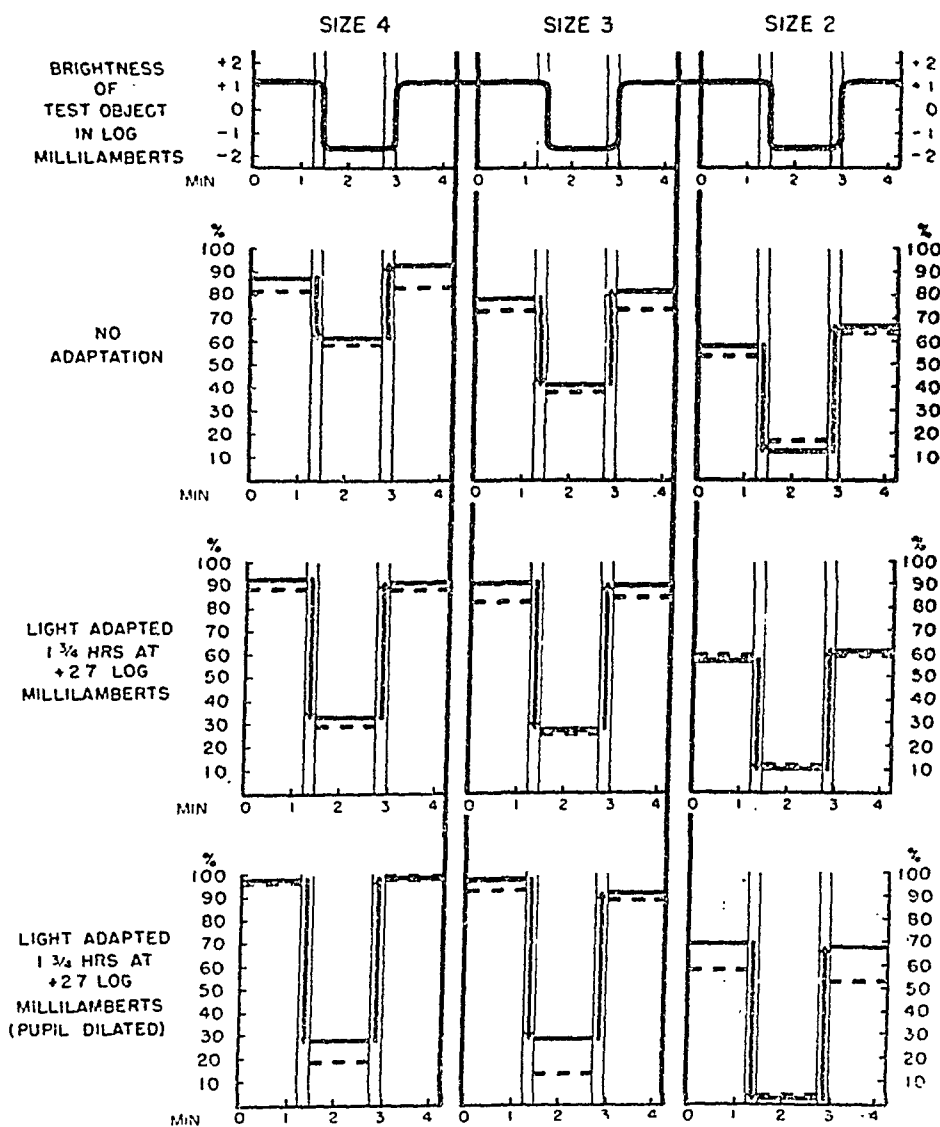


Fig. 1. EFFECT OF CHANGING BRIGHTNESS ON peripheral form perception. Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. The tests were made at  $45^\circ$  on the horizontal meridian of the right eye. Test objects were Landolt circles of 27% contrast with breaks of 4 mm., 3 mm. and 2 mm. Testing intervals between test object sizes were about  $\frac{1}{2}$  min. each. Solid cross bars represent the actual frequency of seeing; dashed cross bars, the subject's certainty of having given correct responses. Experiments began  $\frac{1}{2}$  min. after the end of the light adaptation period. Order of testing progresses from left to right and top to bottom.

for each sequence. The dashed cross bars similarly represent the confidence. The performance is depressed by brightness diminution from  $+1.2$  to  $-1.6$  log mL for all test object sizes and adaptive conditions used. When the original brightness was restored the scores returned to approximately the original level. The loss was greater



when the subjects had been previously light-adapted with normal pupil and still greater after light adaptation with pupils dilated. The statistical significance of the difference between the scores at  $+1.2$  log mL. and those at  $-1.6$  log mL. was checked

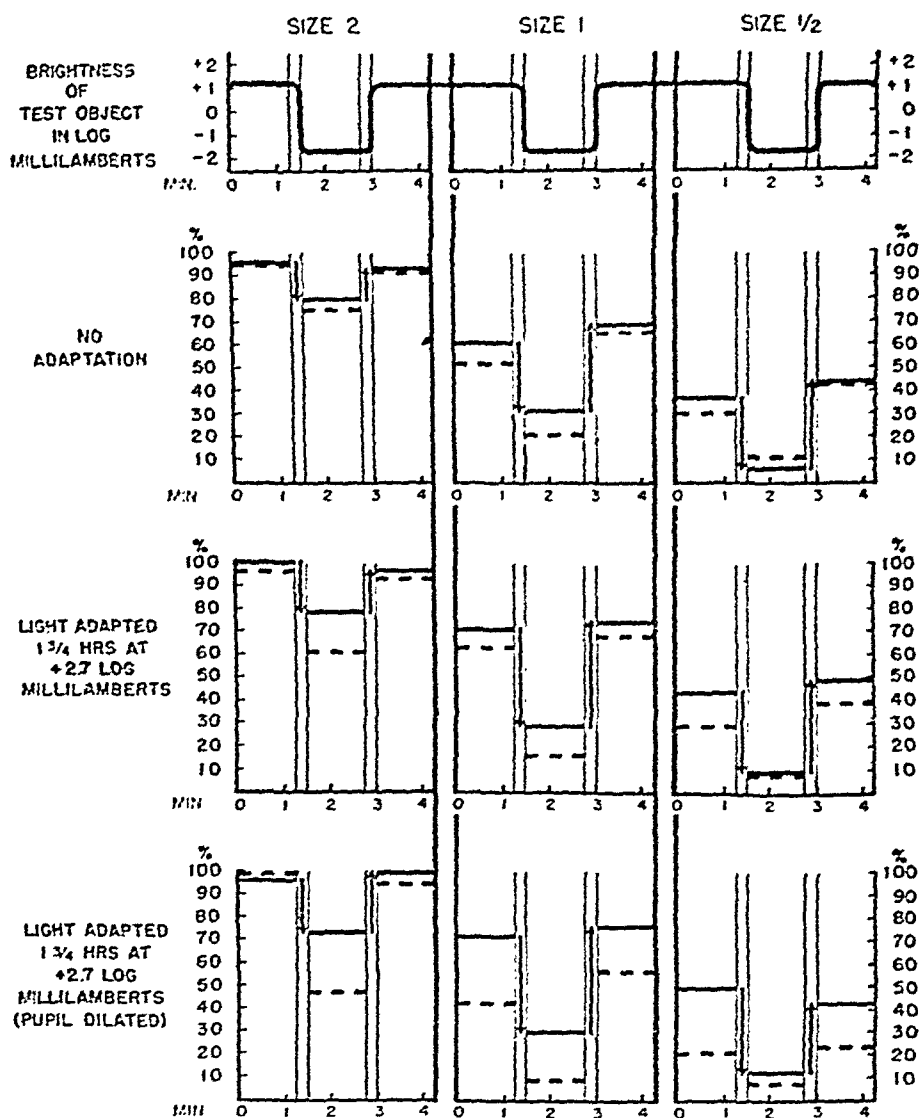


Fig. 2. EFFECT OF CHANGING BRIGHTNESS on peripheral form perception. Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. The tests were made at  $30^\circ$  on the horizontal meridian of the right eye. Test objects were Landolt circles of 97% contrast with breaks of 2 mm., 1 mm. and  $\frac{1}{2}$  mm. Testing intervals between test object sizes were about  $\frac{1}{2}$  min. each. Solid cross bars represent the actual frequency of seeing; dashed cross bars, the subject's certainty of having given correct responses. Experiments began  $\frac{1}{2}$  min. after the end of the light adaptation period. Order of testing progresses from left to right and top to bottom.

by small sample theory formulae (7, p. 59). The first group represented in figure 1 (size 4, no adaptation) has means that are significant at the 2 per cent level of confidence. All of the rest are significant at the one per cent level of confidence. The scores were analyzed to determine whether or not the performance loss in eyes light-

adapted with dilated pupils was significantly greater than the loss sustained by the same subjects when unadapted. In other words, did the light adaptation produce a significantly greater effect? The increase in the depression of the form perception was significant at the one per cent level of confidence with *test objects 4 and 3* and at the 2 per cent level for *size 2*. The mean confidence scores are somewhat less than the mean performance scores and do not show any independent trend.

Figure 2 illustrates the effect of changing brightness on the form perception of high contrast (97%) test objects presented  $30^\circ$  peripherally on the horizontal meridian. Here again, losses have occurred from diminished brightness in all cases. The

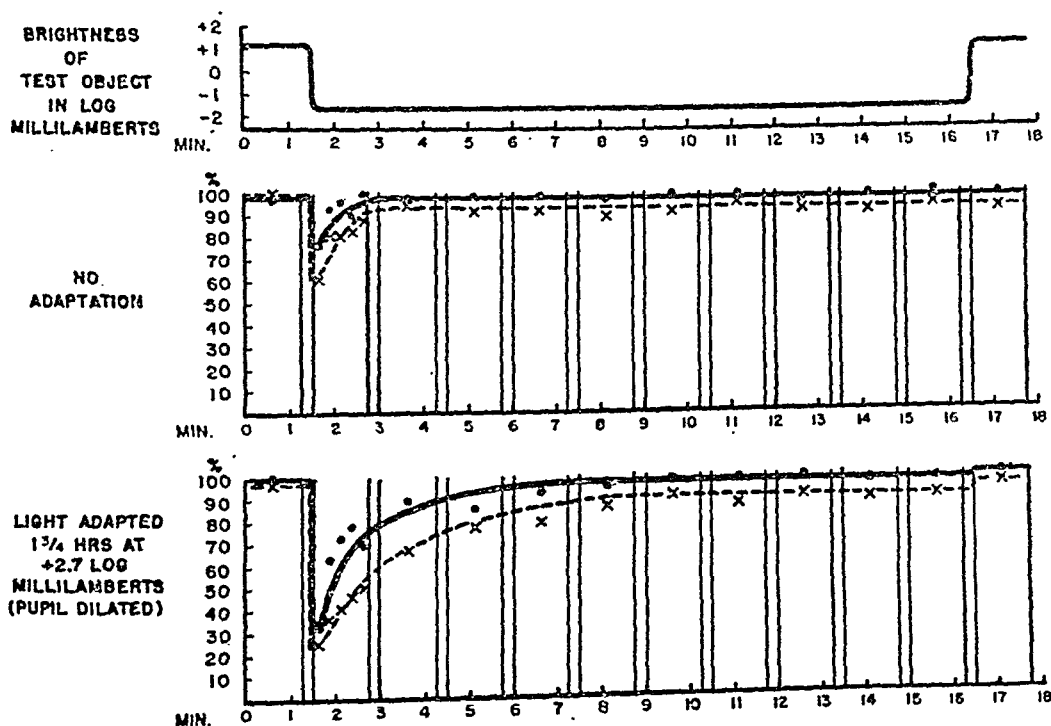


Fig. 3. DURATION OF EFFECT OF CHANGING BRIGHTNESS on peripheral form perception. Average scores of 11 subjects are expressed as the frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. The tests were made at  $30^\circ$  on the horizontal meridian of the right eye. The test object was a Landolt circle of 97% contrast with a break of 2 mm. Dots and solid curves represent the actual seeing; crosses and dashed curves, the subject's certainty of having given correct responses. The scores of the sequence following brightness diminution have been broken down into 15 sec. intervals. The experiment following light adaptation began  $\frac{1}{2}$  min. after the end of the light adaptation period. The curves are drawn by inspection.

differences between the performance scores at  $+1.2$  log ML. and  $-1.6$  log ML. are all significant at the one per cent level of confidence, most of them greatly exceeding the minimal difference required. The results differ from those obtained with low contrast (27%) test objects in that the losses observed after light adaptation, although greater, are not as marked. Statistical analysis of the increase in loss after light adaptation with dilated pupil over that observed in unadapted eyes revealed it to be not significant. The confidence scores show a tendency to separate from the performance scores in the later experiments.

Figure 3 presents the mean scores of 11 subjects in *experiments 11 and 12* which

measured the duration of the effect of diminished brightness on form perception. The second sequence, that immediately following the brightness diminution, is broken down into five 15-second intervals because of the rapid changes taking place. There was little loss and rapid recovery of both performance and confidence when the subjects had received no adaptation. When the subjects had been light-adapted with dilated pupils both performance and confidence losses were measurably greater and

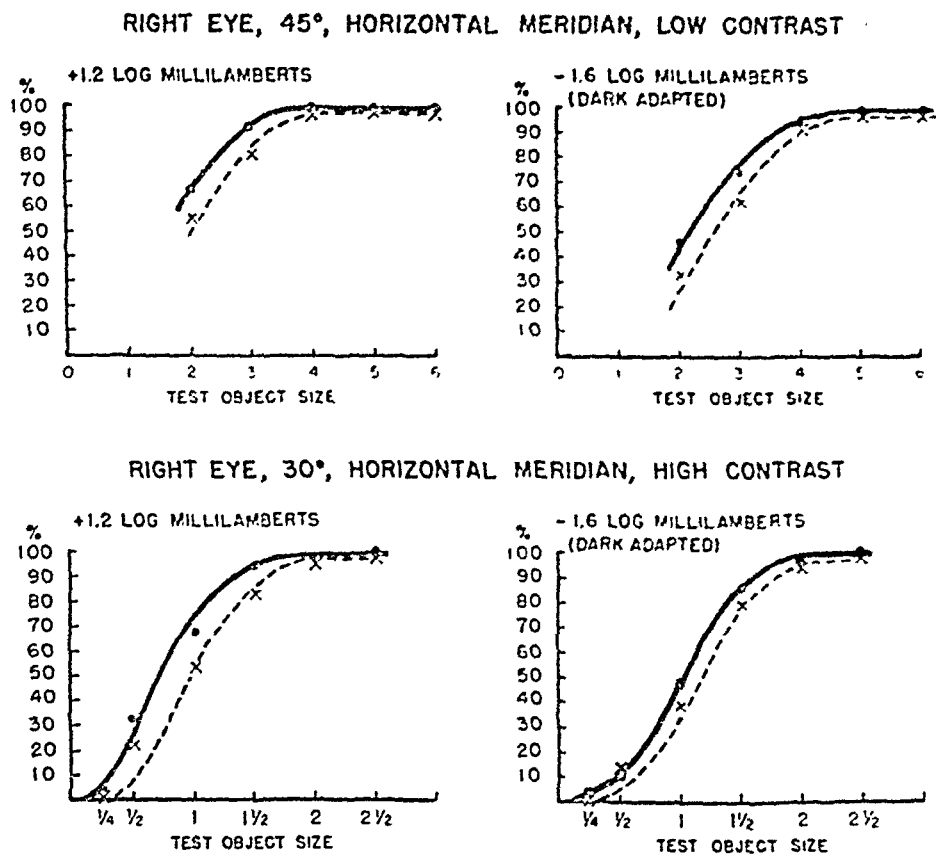


Fig. 4. DIFFERENTIAL THRESHOLD CURVES for peripheral form perception. Average scores of 11 subjects are expressed as the percentile frequency of seeing in sequences of 20 consecutive stimulus presentations over 1 min. 15 sec. Test objects were Landolt circles, the size of which is indicated by the mm. measure of the break. Dots and solid curves represent the actual frequency of seeing; the crosses and dashed curves, the subject's certainty of having given correct responses. The curves are drawn by inspection.

the recovery slower. The confidence seems to lag somewhat, a slight residual effect being noticeable as long as the low brightness was continued.

Figure 4 presents differential threshold curves for peripheral form perception in the same group of 11 subjects. The differential threshold is quite wide, extending from approximately  $\frac{1}{4}$  to 2 with the high contrast test objects, a fact which facilitates the transference of frequency of seeing losses into acuity measures as discussed in a later section of this paper. The confidence scores occupy a position just below the performance curves, which is interpreted as the proper relative value wherever frequency of seeing is not perfect.

## DISCUSSION

Statistically significant losses in form perception have been observed in response to suprathreshold diminution of brightness under all of the experimental conditions that have been systematically investigated. The phenomenon has been observed in all subjects, without individual exception.

Certain differences between the results at  $45^\circ$  with low contrast (27%) test objects and those at  $30^\circ$  with high contrast (97%) test objects are evident. The former show losses in response to diminished brightness which increase significantly as the subject's exposure to bright light increases. A lesser increase occurred with the high contrast (97%) test objects but was not statistically significant. Although the reason for this difference is not clear, it is interesting to note that the low contrast test objects represent a closer approach to field conditions and probably afford a better reflection of practical function.

The differential threshold curves in figure 4 give the performance and confidence scores to be expected for the experimental conditions represented in figures 1, 2 and 3, except for the state of adaptation. In figure 4 the eyes have been adapted to the lower brightness. The level to which any performance or confidence ought to drop because of lesser efficiency at a dimmer illumination is indicated by these curves. The means in figures 1 and 2 and the readings on the differential threshold curves of figure 4 are almost the same for the higher brightness for sizes where the frequency of seeing is high. The frequency of seeing for the same test object size at the lower brightness in figure 4 is nearly the same, indicating a similar anticipated performance at the lower brightness. In spite of this there is a marked drop in frequency of seeing upon diminished brightness when the eye is not adapted to the lower brightness. For example, in figure 1, size 4, after adaptation at  $+2.7 \log \text{ m.L.}$  for  $1\frac{3}{4}$  hours with pupils dilated the performance loss of from 98 per cent to 30 per cent greatly exceeds that indicated by the differential threshold curves. On the latter the loss does not exceed 5 per cent. The performance losses exceed the anticipated losses in all cases and suggest a temporary impairment, likely of adaptive nature.

The above suspicion is largely confirmed by the experiments reported in figure 3, which measured the duration of the perceptual loss and graphed its recovery to the anticipated value. When no adaptation preceded the experiment the loss was small and the recovery rapid, being complete in about  $1\frac{1}{2}$  minutes. The same technique following light adaptation with dilated pupils resulted in a greater loss which took about four times as long to recover. These curves clearly indicate the adaptive nature of the changes. In both experiments represented in figure 3 the frequency of seeing returns almost to the level for  $+1.2 \log \text{ m.L.}$  while the brightness is still at  $-1.6 \log \text{ m.L.}$ , a circumstance predicted by the differential threshold curves of figure 4.

An estimate of changes in acuity can be made from data presented as frequency of seeing. When brightness is diminished the frequency of seeing falls to a level characteristic of a smaller test object. By assuming that the test object size indicated by the lowered frequency of seeing is to the test object size actually used as the latter is to the size required for comparable performance under the new conditions, a measure of the loss in terms of test object size can be calculated. The data in figure

3 for the first 15 seconds at  $-1.6 \log \text{mL.}$  after light adaptation with pupils dilated will serve as an example. At the higher brightness frequency of seeing was 98.8 per cent, only to drop to 30 per cent upon diminished brightness for the 15-second interval mentioned above. By reference to figure 4 it will be seen that 30 per cent frequency of seeing is normally given by size  $\frac{1}{2}$  (approx.). Then,  $\frac{1}{2}$  is to 2 as 2 is to  $x$ ; size 8 would be required for the original frequency of seeing during the first 15 seconds of the lower brightness. Since the stated test object size is the linear measure of the break in the circle, the areas of the two test objects are related as 1 is to 16. It then follows that, other conditions being the same, a retinal area 16 times the size of that originally stimulated would be required for responses of equal efficiency. The recovery process equates the sizes in about six minutes, when the acuity has reached the fully adapted level. An effect of comparable magnitude but probably of longer duration is evident with low contrast (27%) test objects of sizes 4 and 3. In figure 1 after light adaptation both with and without dilated pupils the frequencies of seeing losses were comparable. These scores cover the mean performance during the first  $1\frac{1}{4}$  minutes in the low brightness as compared to the first 15 seconds in the cited example. This method of deriving relative acuity from frequency of seeing, although crude with the present technique and data, serves to indicate the magnitude of the changes in terms convertible into accepted expressions of visual acuity.

The brightnesses used were purposely chosen at levels above those customarily associated with adaptive phenomena. The lower brightness,  $-1.6 \log \text{mL.}$ , is supra-threshold for both rods and cones according to the data of Hecht (1) and Winsor and Clark (2) for the unadapted eye. This was confirmed subjectively among the present group of subjects and held true even after the light adaptation. Nevertheless the results are clearly of an adaptive nature. They closely resemble the gradual development of form acuity during the process of dark adaptation previously reported by the writer (8) for lower brightnesses. In this previous study unadapted subjects were suddenly deprived of light except for that from the test object illuminated at a low scotopic level. The time required for them to perceive the light on the test object was recorded and represented attainment of the absolute threshold for that brightness. Then their peripheral form acuity was measured by a limiting method requiring 80 to 100 per cent frequency of seeing. The form acuity was found to develop gradually to its maximal level in from 2 to 17 minutes after light was perceived. The responses of the present experiments probably represent an expression of the same phenomenon except that the brightnesses used are higher and never cross the absolute threshold. The maintainance of brightness within suprathreshold levels does not prevent the deterioration of form perception.

Interpretation of the results reported here should not be restricted to the retinal areas actually tested. Comparable effects should be expected to occur in all portions of the retina and should be anticipated in terms of the adaptive characteristics of the area in question and the adaptive state of the individual. No data can be offered at present concerning the upper limit of brightness at which significant deterioration of form perception can be produced, but it is probable that it occurs at higher brightnesses than those at which it has already been detected. The cumulative effect of regular exposure to sunlight already reported by Hecht (3) and the greater effect

after preadaptation to bright light reported here encourage such a view. The brightness of the preadapting light used in the present experiments is scarcely an approach either in brightness or duration to that experienced by individuals living an outdoor life in sunny weather. In such individuals in whom the absolute threshold has been raised with concomitant sluggish adaptation, the diminishing brightness of the post-sunset hours may well approach the absolute threshold closely enough to produce significant deterioration of form perception while considerable subjective brightness still persists. The losses are primarily adaptive ones which can and doubtless do occur at levels of brightness higher than those currently associated with adaptive phenomena.

#### SUMMARY

The effect of sudden suprathreshold diminution of brightness from  $+1.2 \log \text{mL.}$  to  $-1.6 \log \text{mL.}$  on peripheral form perception was measured in 11 subjects. The above brightness diminution causes a loss in frequency of seeing which is significant at the one per cent level of confidence with both high contrast (97%) and low contrast (27%) test objects. The loss due to diminished brightness is significantly greater after the eyes have been preadapted to a brightness of  $+2.7 \log \text{mL.}$  with dilated pupil when low contrast (27%) test objects are used. When high contrast (97%) test objects are used the loss is greater after preadaptation as above but not significantly so.

The duration as well as the extent of the loss is increased by preadaptation to  $+2.7 \log \text{mL.}$  with pupil dilated. The subject's confidence in having seen correctly under these conditions is somewhat less than the actual seeing ability but maintains a fairly constant relation to it. The changes observed are of a primarily adaptive nature and are not traceable to different acuities for different brightnesses.

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# STUDY OF THE EXCHANGE OF OXYGEN AND CARBON DIOXIDE IN THE SUPRAGLOTTIC PORTION OF THE RESPIRATORY DEAD SPACE

MORTON GALDSTON AND SEYMOUR A. HORWITZ

*From the Research Service, Third (New York University) Medical Division, The Goldwater Memorial Hospital, Welfare Island, and the Department of Medicine, New York University College of Medicine*

NEW YORK CITY

THE question as to whether an exchange of oxygen and carbon dioxide occurs across the mucous membranes of the respiratory dead space has concerned physiologists for many years. Henderson believed that carbon dioxide diffuses across the mucous membrane of the bronchial tree because he noted that the ratio of carbon dioxide output to oxygen consumption ( $RQ$ ) in expired air is greater than in alveolar air (1). Subsequently, it was demonstrated by gas equilibration studies carried out over several minutes that carbon dioxide and oxygen can diffuse across the mucous membrane of the oral portion of the respiratory dead space (2).

Haldane and Priestley (3) and Krogh and Lindhard (4) did not believe that gas transfer occurred in the respiratory dead space. Haldane and Priestley postulated that the difference between the respiratory quotient of expired and alveolar air is due to unequal ventilation of the alveolar ducts and atria of the lungs and to a greater rate of diffusion of carbon dioxide than oxygen through tissues (3), whereas Krogh and Lindhard ascribed it to errors inherent in the Haldane-Priestley method of collecting alveolar air (5). Neither of these views has been established (6-8). However, in calculating the various pulmonary air fractions it has been generally assumed that gaseous exchange does not occur in the respiratory dead space.

The studies to be reported were undertaken to ascertain by direct measurement whether rapid transfer of oxygen and carbon dioxide occurs in the respiratory dead space and if so what bearing it might have on aspects of pulmonary function calculated from analyses of expired and alveolar air.

## METHODS

The studies were carried out in 2 healthy adults at rest in a recumbent posture one to two hours following breakfast. A small rubber catheter with two holes, each 3 mm. in diameter within 2 cm. of the distal end, was threaded through one nostril until the distal hole was visible just below the uvula. A standard metal three-way stopcock inserted into the proximal end of the rubber catheter served to join it to a mercury gas sample tube evacuated to approximately 40 cc. and to a 20-cc. luer-lok syringe filled with room air. The nostrils were occluded securely with a nose clip. With a rubber mouth piece in place, the subject inhaled room air and exhaled into a Tissot spirometer. The rate and depth of respiration were

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Received for publication October 4, 1948.

recorded on an ink-writing drum attached to the spirometer. During the inspiration just preceding the collection of an air sample the catheter, which had a capacity of 2 cc., was flushed with 15 cc. of room air from the syringe attached to the three-way stopcock. At the moment when the subject completed the inspiration he was ordered to hold his breath and perform a Valsalva maneuver. At the same time he occluded the open end of the rubber mouth piece with his tongue. These maneuvers assured collection of air samples from the supraglottic portion of the respiratory dead space. Air samples were then collected in the evacuated mercury tube either immediately (within 1 or 2 sec.) or at any desired interval up to 40 seconds. In several instances air samples were gently drawn in four 10 cc. portions into an air-tight syringe and transferred to a mercury tube to learn whether the strong suction exerted by the evacuated mercury tube introduced a source of error.

The momentary pause in respiration, necessitated by the collection of air from the supraglottic portion of the respiratory airway, did not alter the pattern of respiration during the few minutes that it was recorded immediately thereafter. In 11 instances expired air was collected for approximately three minutes prior to collection of the supraglottic air sample and for three to four minutes thereafter.

Gas samples were analyzed in duplicate in a Haldane analyzer and results were compared with the composition of room air, which was found to contain consistently  $20.93 \pm 0.04$  volumes per cent oxygen and  $0.03 \pm 0.03$  volumes per cent carbon dioxide. The room was well ventilated with outdoor air.

### RESULTS

A definite but small rise in carbon dioxide tension and fall in oxygen tension of inspired room air occurs in the supraglottic portion of the respiratory dead space, when the breath is held for one to two seconds after the end of inspiration while the subject performs a Valsalva maneuver (table 1). The average rise in carbon dioxide tension in 30 observations was 3.7 mm. with a standard deviation of 1.8 mm. Hg. The average fall in oxygen tension for a similar number of observations was 3.7 mm. Hg, with a standard deviation of 2.3 mm. Hg. The close agreement between the results obtained from analysis of air samples drawn in one 40-cc. portion into an evacuated mercury tube and those of similar volume drawn by syringe in 10 cc. portions indicates that with the former method, which was used in most instances, air was not drawn from poorly ventilated stagnant areas. When the breath is held for progressively longer periods, the proportion of carbon dioxide increases at a nearly steady rate whereas all of the decrease in the proportion of oxygen appears to occur within at most 10 seconds (fig. 1). Though there is considerable daily variation in the rate of exchange of these gases for similar periods of breathholding, the trend is consistent and it is not related to the depth of breathing, minute volume of respiration or respiratory quotient based on analysis of expired air.

### DISCUSSION

Analyses of air samples drawn from the respiratory dead space at the end of either inspiration or expiration should indicate whether or not the dead space is an inert



TABLE 1. DATA CONCERNING STUDIES ON EXCHANGE OF CARBON DIOXIDE AND OXYGEN IN THE SUPRAGLOTTIC PORTION OF THE RESPIRATORY AIRWAY WITHIN 1 TO 2 SECONDS AFTER THE END OF INSPIRATION

OBSERVATION	TIDAL AIR <sup>1</sup>	VENTILATION RATE <sup>1</sup>	SUPRAGLOTTIC AIR				EXPIRED AIR		RQ
			CO <sub>2</sub>	O <sub>2</sub>	pCO <sub>2</sub>	pO <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	
			Content		Rise	Fall	Content		
			vol. %		mm. Hg. <sup>2</sup>		vol. %		
1	401	6.4	0.57	20.49	3.9	3.0	3.87	16.88	.94
2	352	8.5	0.53	20.45	3.6	3.4	3.32	17.39	.93
3	374	8.6	0.77	20.29	5.3	4.3			
4	470	5.2	0.34	20.59	2.2	2.5	4.15	16.43	.90
5	570	5.1	0.23	20.80	1.4	0.7			
6	410	3.3	0.43	20.65	2.8	1.8			
7	285	4.6	0.33	20.63	2.2	2.2	3.35	17.22	.90
8	280	3.9	0.42	20.56	2.8	2.6			
9	642	6.4	0.57	20.47	3.9	3.1			
10	708	5.7	0.30	20.64	2.0	2.2	4.06	16.29	.84
11	764	5.3	0.72	20.19	4.9	5.4	3.99	16.01	.77
12	821	5.7	0.44	20.49	2.9	3.2			
13	622	5.0	0.35	20.66	2.3	1.9			
14	697	5.6	0.37	20.60	2.4	2.3	4.16	16.17	.84
15	693	4.9	0.28	20.71	1.8	1.5			
16	662	5.3	0.28	20.66	1.8	2.0			
17	526	4.7	0.30	20.71	1.9	1.5			
18	681	6.1	0.19	20.75	1.1	1.3			
19	880	7.0	0.98	19.95	6.8	7.2	3.55	16.91	.85
20	680	4.1	0.94	20.00	6.6	6.7			
21	870	4.4	0.74	20.27	5.1	4.7			
22	740	5.2	0.86	20.01	6.0	6.9	3.94	16.14	.78
23	750	5.3	1.19	19.56	8.3	10.3			
24	880	6.2	0.53	20.42	3.6	3.7	3.70	16.68	.83
25	864	5.2	0.37	20.59	2.4	2.4			
26	896	5.4	0.53	20.39	3.6	3.9			
27	670	4.0	0.56	20.36	3.8	4.1			
28	838	5.0	0.80	20.00	5.5	7.0			
29	902	6.3	0.33	20.67	2.1	1.9			
30	948	6.5	1.12	19.71	7.8	8.7	3.79	16.58	.83
Average.....					3.7	3.7			
Standard Deviation.....					1.8	2.3			

<sup>1</sup> Expressed at body temperature, ambient pressure, saturated.

<sup>2</sup> Calculated from inspired air containing  $20.93 \pm 0.04$  vol. % O<sub>2</sub> and  $0.03 \pm 0.03$  vol. % CO<sub>2</sub> at prevailing barometric pressure less aqueous tension, 47 mm. Hg.

conduit. Collection of a sample of air at the end of inspiration offers however a better standard of comparison (inspired room air) than does expiration (expired air).

It seems likely that the gas exchange occurs across the mixed glandular secretions which bathe the supraglottic area. By far the greatest portion of the secretions

comes from the salivary glands. It has been demonstrated that the nitrogen (9) and carbon dioxide content of saliva reflect closely the level of these gases in blood, that mixed saliva generally has a  $pH$  of 6.5 to 6.8 and that it contains oxygen (10). The circumstances most favorable for transfer of these gases, between the layer of mixed secretions and the air in the respiratory airway, would appear to be present at the end of inspiration when the tension of carbon dioxide in the airway is at its lowest level and the tension of oxygen is at its highest.

These studies establish what had previously been inferred by Henderson concerning output of carbon dioxide into the respiratory dead space (1). They also demonstrate that oxygen uptake of approximately the same magnitude as carbon dioxide

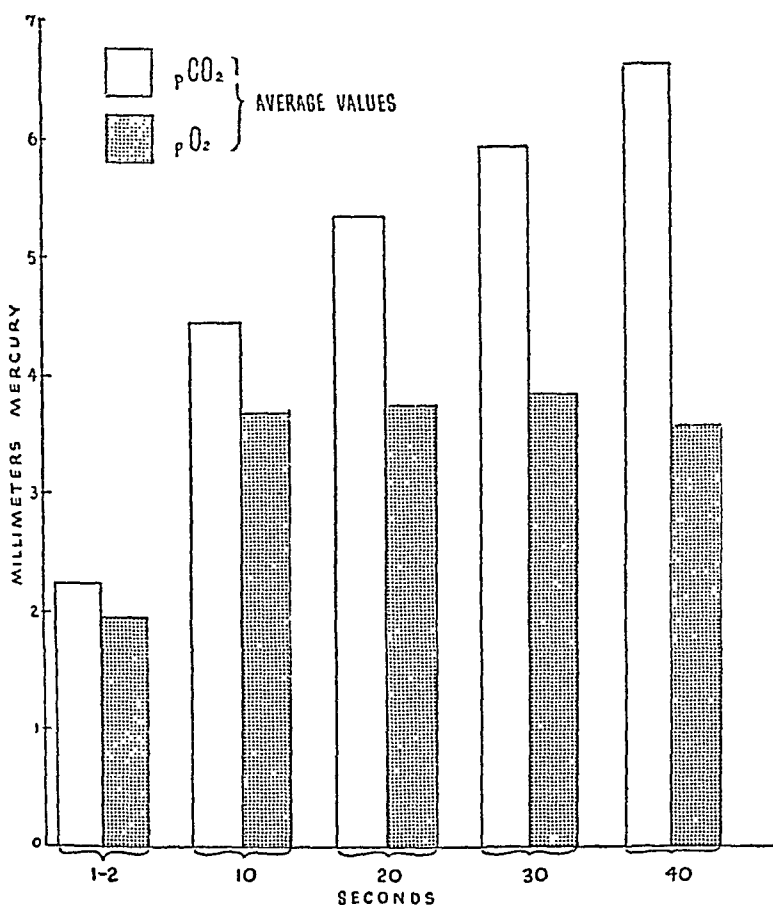


Fig. 1. GAS EXCHANGE in supraglottic portion of respiratory airway. Average tension values for breathholding are based on serial determinations carried out on different days. There were 6 observations at 1-2 sec., 5 at 10, 20 and 30 sec. and 4 at 40 sec. The range of values in mm. Hg of carbon dioxide and oxygen tension respectively was: at 1-2 sec., 1.7-2.8, 1.5-2.9; 10 sec., 3.7-5.9, 2.9-5.3; 20 sec., 4.5-7.1, 2.7-5.9; 30 sec. 5.0-8.4, 2.2-6.7; 40 sec., 6.5-6.9, 2.1-5.1.

output occurs in the supraglottic portion of the respiratory dead space during quiet respiration. The transfer of these gases cannot, therefore, account for a difference between the respiratory quotient as measured in expired and alveolar air since the ratio of exchange is approximately one.

An average rise in carbon dioxide tension and fall in oxygen tension of 3.7 mm. Hg occurs in the supraglottic portion of the respiratory airway (table 1). It is generally believed that in healthy subjects at rest, the carbon dioxide tension of inspired air rises 40 mm. Hg (from 0 to 40 mm. Hg) and oxygen tension of inspired air falls 50 mm. Hg (from 150 to 100 mm. Hg) in the alveoli. This fraction represents approximately 9 per cent of the total carbon dioxide output and 7 per cent of the total oxygen consumption of the total gaseous exchange in the lungs.

TABLE 1. DATA CONCERNING STUDIES ON EXCHANGE OF CARBON DIOXIDE AND OXYGEN IN THE SUPRAGLOTTIC PORTION OF THE RESPIRATORY AIRWAY WITHIN 1 TO 2 SECONDS AFTER THE END OF INSPIRATION

OBSERVA- TION	TIDAL AIR <sup>1</sup>	VENTILA- TION RATE <sup>1</sup>	SUPRAGLOTTIC AIR				EXPIRED AIR		RQ
			CO <sub>2</sub>	O <sub>2</sub>	pCO <sub>2</sub>	pO <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	
			Content		Rise	Fall	Content		
			vol. %		mm. Hg. <sup>2</sup>		vol. %		
1	401	6.4	0.57	20.49	3.9	3.0	3.87	16.88	.94
2	352	8.5	0.53	20.45	3.6	3.4	3.32	17.39	.93
3	374	8.6	0.77	20.29	5.3	4.3			
4	470	5.2	0.34	20.59	2.2	2.5	4.15	16.43	.90
5	570	5.1	0.23	20.80	1.4	0.7			
6	410	3.3	0.43	20.65	2.8	1.8			
7	285	4.6	0.33	20.63	2.2	2.2	3.35	17.22	.90
8	280	3.9	0.42	20.56	2.8	2.6			
9	642	6.4	0.57	20.47	3.9	3.1			
10	708	5.7	0.30	20.64	2.0	2.2	4.06	16.29	.84
11	764	5.3	0.72	20.19	4.9	5.4	3.99	16.01	.77
12	821	5.7	0.44	20.49	2.9	3.2			
13	622	5.0	0.35	20.66	2.3	1.9			
14	697	5.6	0.37	20.60	2.4	2.3	4.16	16.17	.84
15	693	4.9	0.28	20.71	1.8	1.5			
16	662	5.3	0.28	20.66	1.8	2.0			
17	526	4.7	0.30	20.71	1.9	1.5			
18	681	6.1	0.19	20.75	1.1	1.3			
19	880	7.0	0.98	19.95	6.8	7.2	3.55	16.91	.85
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It seems likely that the gas exchange occurs across the mixed glandular secretions which bathe the supraglottic area. By far the greatest portion of the secretions

comes from the salivary glands. It has been demonstrated that the nitrogen (9) and carbon dioxide content of saliva reflect closely the level of these gases in blood, that mixed saliva generally has a  $pH$  of 6.5 to 6.8 and that it contains oxygen (10). The circumstances most favorable for transfer of these gases, between the layer of mixed secretions and the air in the respiratory airway, would appear to be present at the end of inspiration when the tension of carbon dioxide in the airway is at its lowest level and the tension of oxygen is at its highest.

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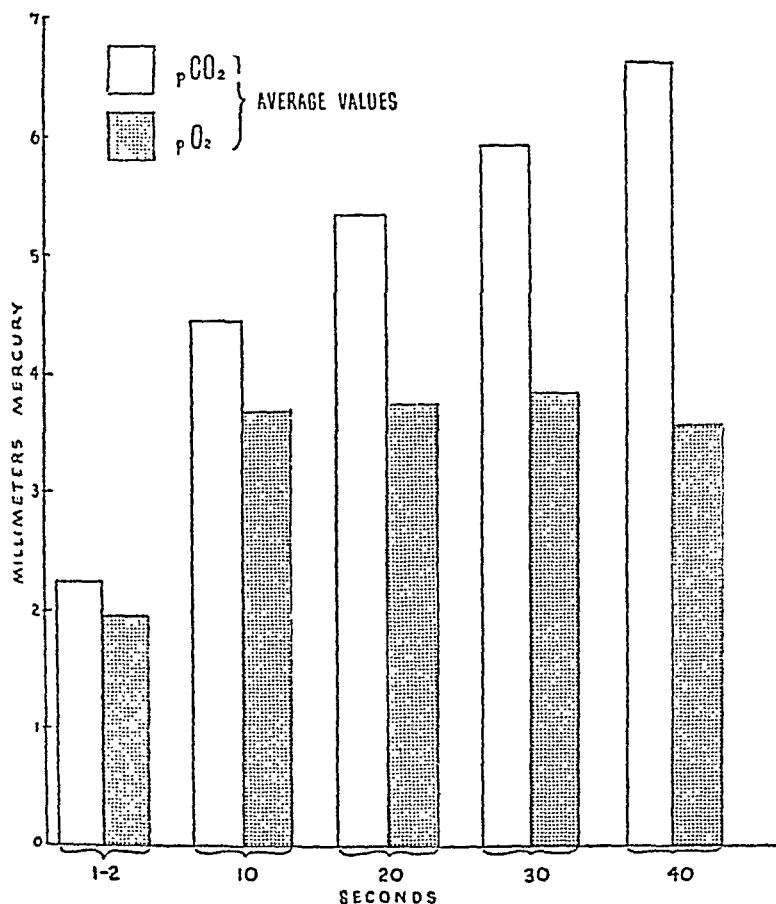


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The studies during breathholding bring out a distinct difference between exchange of carbon dioxide and oxygen (fig. 1). Carbon dioxide concentration increases steadily during breathholding whereas the concentration of oxygen falls only within approximately the first 10 seconds. The findings are consistent with Henderson's observation that with breathholding there is a greater decrease in the size of the respiratory dead space calculated for carbon dioxide than for oxygen (1). It would appear that the difference in exchange is due to the much more rapid rate of diffusion of carbon dioxide through body tissues and fluids and to the limitations imposed on oxygen exchange by its absorption coefficient in the mixed glandular secretions which bathe the supraglottic portion of the respiratory airway.

#### SUMMARY

An exchange of carbon dioxide and oxygen of approximately the same magnitude occurs in the supraglottic portion of the respiratory dead space within one to two seconds after the end of inspiration in healthy subjects at rest. This exchange does not account for any difference reported in the *RQ* of expired and alveolar air. When the breath is held at the end of inspiration for progressively longer periods of time, the carbon dioxide concentration increases at a nearly steady rate, whereas the oxygen concentration falls only during the first 10 seconds.

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# THE AMERICAN PHYSIOLOGICAL SOCIETY PROCEEDINGS

## FIRST FALL MEETING

September 16, 17, 18, 1948

The following are abstracts of papers presented at the First Fall Meeting of The American Physiological Society, held at the University of Minnesota, Minneapolis, and at the Mayo Foundation and Mayo Clinic, Rochester, Sept. 16-18, 1948.

*Hormonal alterations in men exposed to heat and cold stress.* RICHARD A. BADER, HAROLD J. STEIN, JOHAN W. ELIOT AND DAVID E. BASS (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass.

Hormonal alterations were measured in three men who were subjected to heat and cold stress utilizing intermittent exposures to heat (107°F. dry bulb; 89°F. wet bulb) and to cold (-20°F.) as stress stimuli. The number of circulating eosinophils, which can be depressed by anterior pituitary adrenocorticotrophic hormone (ACTH) or Compound F, were significantly reduced in daily counts during (1) exercise, (2) a combination of heat and exercise, and (3) a combination of cold and exercise, compared to a baseline period without stress. Adrenal cortical reserve was assessed by the response to ACTH in the manner proposed by Forsham *et al.* (*J. Clin. Endocrinol.* 8: 15, 1948), following the heat, and again following the cold exposures. These studies showed a slightly 'abnormal response' in one individual and an essentially 'normal response' in the other two subjects. This suggests that the heat and cold stresses were not sufficient to deplete the adrenal cortical reserve as measured by ACTH. The heat stress was nevertheless, sufficient to produce acclimatization, although definite acclimatization to cold was not demonstrated. No significant changes were found during any of the stress periods in the following indices: urinary uric acid-creatinine ratio, urinary 17-keto-

steroid excretion, absolute lymphocyte counts, and basal metabolism.

*Influence of various diuretic substances on the renal excretion of electrolytes in the dog.* DAVID BALDWIN, A. P. CROSELY, JR., AND P. J. TALSO (introduced by ROBERT W. CLARKE). Medical Dept. Field Research Laboratory, Fort Knox, Ky.

Unanesthetized dogs have been studied during the administration of four diuretics. Inulin and PAH clearances were measured. Sodium and potassium were determined by flame photometry. Intravenous hypertonic saline infusion, which raised the serum sodium and glomerular filtration, reduced tubular sodium reabsorption from 98% to 80% of the filtered amount. The fractional tubular reabsorption of potassium was much more severely depressed than that of sodium. Intravenous aminophyllin raised glomerular filtration but did not alter serum sodium. Tubular reabsorption of sodium rose in proportion to filtration. Potassium excretion was augmented proportionally more than sodium, tubular reabsorption of potassium remaining constant. Mersalyl given by slow intravenous injection caused the tubular reabsorption of sodium to fall relative to the slightly elevated rate of glomerular filtration and to the tubular load. In spite of a slight increase in the tubular load of potassium the rate of its reabsorption fell. The fraction of filtered potassium which the tubules reabsorbed was reduced

but by a different means than that shown with aminophyllin. Intravenous hypertonic mannitol infusion somewhat decreased filtration, and reduced serum sodium and potassium slightly. Sodium reabsorption also fell, to a greater extent than the load, and the fraction of the latter which was reabsorbed was significantly depressed. There was a great fall in potassium reabsorption, 25% of the load being excreted. When more sodium is filtered through the glomeruli it is reabsorbed by the tubules in preference to potassium. The diuretics studied decreased the relative tubular reabsorption of potassium far more than of sodium.

*Total gastrectomy in the rat.* DONALD C. BALFOUR, JR., AND GEORGE M. HIGGINS. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

The effect of total gastrectomy has been studied extensively in the larger animals such as the dog, cat, pig, monkey and also in man. It has been shown that after gastrectomy it is difficult to maintain normal weight, a secondary anemia will usually develop and a homogeneous osteoporosis can often be demonstrated. Nutritional studies have been done on the totally gastrectomized rat. It is found that the gastrectomized rat is incapable of maintaining adequate food intake over a long period of time. By selestine diet studies it was shown that the gastrectomized rat selects a diet which is lower in protein content than the diet of the normal rat. Replacement therapy with gastric extracts does not improve the nutrition of the animal and injections of liver extract and vitamin B<sub>1</sub> improve it only temporarily.

*Influence of prolonged administration of thiouracil on growth and metabolism.* S. B. BARKER. Dept. of Physiology, State Univ. of Iowa, Iowa City.

Fertility is markedly reduced in adult female rats rendered hypothyroid by thiouracil inhibition of the thyroid gland, an observation which may be explained on the basis of altered estrus cycles. Cretinoid young have been obtained by procedures involving early depression of thyroid function with thiouracil, and have been maintained at a stage of incomplete development for periods up to 16 months by continuing the thiouracil administration. These animals have large, colloid-free thyroids, low plasma PI values and B.M.R.'s lowered by 20 to 35%, further confirming the lowered thyroid activity. Even after 16 months of depressed thyroid function, withdrawing the thiouracil results in a temporary renewal of growth, plus recovery of the lost reproductive capacity in some of the animals. The long-inhibited gland recovers its ability to store thyroglobulin and to release into the blood stream

enough thyroid hormone to cause the B.M.R. to return to normal even though the plasma level of hormone is not brought completely to normal. Reproductive activity is rapidly restored, and apparently normal offspring are produced by females with weights plateaued at less than half the normal value.

*Relation of the adrenal glands to the renin concentration of the canine kidney.* HERBERT E. BESSINGER (by invitation) AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Study was made of the renin concentration of the kidneys of 21 dogs, three to eight weeks following unilateral renal artery constriction. Three of the dogs were bilaterally adrenalectomized and maintained by salt therapy only. The condition of adrenal insufficiency did not appear to reduce the renin concentration of the renal artery constricted kidney determined four weeks after unilateral constriction, but appeared to favor renin return to the contralateral non-constricted kidney at which time it normally contains no (or nearly no) renin. The absence of both adrenal glands in 3 bilaterally adrenalectomized dogs with unilateral renal artery constriction receiving maintenance doses of desoxycorticosterone acetate (Schering) does not alter the low or absent renin content of the contralateral, non-constricted kidney six to eight weeks after renal artery constriction. The injection of DCA in nine unilaterally renal artery constricted dogs for periods of three to six weeks following renal artery constriction significantly decreased the extractable renin from the constricted kidney, but did not change the low or absent renin concentration of the contralateral, non-constricted kidney either three or six weeks after unilateral renal artery constriction. The renin was significantly decreased in the kidneys of three normal dogs receiving DCA for five weeks.

*Renal tubular excretion of N-methylnicotinamide by the dog.* KARL H. BEYER, HORACE F. RUSSO, S. R. GASS, KATHARINE B. MILLER AND ALICE A. AMAN. Dept. of Pharmacology, Medical Research Division, Sharp and Dohme, Inc., Glenolden, Pa.

In the abstracts of communications of the XVII International Physiological Congress (p. 217, 218) Sperber indicated that N-methyl-nicotinamide (NMN) was excreted by the tubules of the chicken kidney. Since this is the first instance of the tubular excretion of a base, other than possibly creatinine, we undertook to examine its excretion in dogs. Using the analytical method of Huff and Perlzweig (*J. Biol. Chem.* 167: 157, 1947) we found that the renal clearance and the extraction of N-methyl nicotinamide from the renal blood

flow was greater than for creatinine, indicating tubular excretion of the NMN. As the plasma concentration of NMN was elevated its clearance was depressed to approach glomerular filtration rate. The clearance of NMN was not depressed by p-aminohippurate (PAH) or by caronamide. Therefore it was concluded that the tubular transport mechanism for NMN was separate and distinct from the mechanism responsible for the tubular excretion of PAH. Also, that caronamide which is capable of inhibiting the organic acid tubular excretory mechanism is not capable of inhibiting the excretion of this strong base. Apparently there exists in the mammalian tubules (dogs) a transport mechanism for the renal tubular elimination of organic bases.

*Thresholds for production of seizures by photic stimulation in man.* R. G. BICKFORD (introduced by E. H. LAMBERT). Dept. of Physiology, Mayo Foundation, Rochester, Minn.

Previous studies in this laboratory have shown that approximately five % of epileptic patients are photosensitive. Minor seizures may be induced in this group of patients by subjecting them to flickering light of the requisite intensity. By controlling the intensity of the light stimulus it is possible to make measurements of the convulsive threshold. Three light sensitive subjects were used. They were subjected to 15-second periods of stimulation by light from a reflecting type photoflood bulb at a distance of 3 feet. The beam was interrupted nine times per second by a rotating  $\frac{3}{4}$  blackened sectoring disc. The intensity of the light stimulation was controlled by changing (by means of a variable transformer) the voltage across the photoflood bulb in 10 volt steps between 20 and 110 volts. This represents a brightness range from 3 to 1200 ft candles. A 15-second rest period is given between each stimulation. The convulsive threshold is the lowest light intensity (or voltage) which will induce a seizure within the stimulus period. The occurrence of the seizure is detected by observation of clinical signs and electroencephalographic recording of the seizure discharge. In the resting patient the convulsive threshold may remain constant for several hours if the testing is not repeated at less than 15-minute intervals. Occasionally a slowly progressive increase or decrease in the threshold is observed. Barbiturate drugs in non-hypnotic doses raise the convulsive threshold. Amytal, which is the most effective of the group, may prevent seizures occurring at the highest light intensity. Tri-dione is less effective. Bromides even in hypnotic doses are without effect. Benzedrine and metrazol lower the convulsive threshold.

*Inhibitors of gastric secretion occurring in gastric juice and gastric mucin.* CHARLES M. BLACKBURN AND CHARLES F. CODE. Mayo Foundation, Rochester, Minn.

Using an assay method developed by Code, Blackburn, Ratke and Livermore for the determination of inhibitors of gastric secretion, a study has been made of the inhibitors occurring in human gastric juice. The observation by Brunshwig and his co-workers that there is an inhibitor in achlorhydric gastric juice of patients who have pernicious anemia has been confirmed. The observation of Brunshwig and his associates that the inhibitor is present in achlorhydric gastric juice from patients without other demonstrable changes in gastric function has also been confirmed. Brunshwig and his collaborators tested for the inhibitor by injection of the juice or extracts of the juice into dogs with gastric pouches when these were secreting in response to the ingestion of a meal. We have found that the gastric secretory inhibitor present in human gastric juice also inhibits the secretion of Heidenhain pouches in dogs when these pouches are stimulated by repeated injections of histamine. In a search for the source of the inhibitors present in human gastric juice, we have tested gastric mucin and gastric pepsin. We have found that intravenous injection of gastric mucin produces a pronounced reduction in the secretion of juice from Heidenhain pouches in dogs when these are stimulated by histamine. The preparations of pepsin so far tested have not contained an inhibitor.

*Stimulating effect of carbohydrate, fat and protein meals on duodenal secretion.* D. BLICKENSTAFF (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Carbohydrate, fat and protein test meals were fed to mongrel dogs, some with transplanted pouches of the upper duodenum, fashioned according to the method used by Florey and Harding, and Sonnenschein, Grossman, and Ivy, and others with a similar preparation except that the pouch was opened to form a rectangular flap. The following foodstuffs were used as test meals: 50 grams of dextrose in 100 cc. of water, 50 cc. cottonseed oil, and 50 grams of lean beef. The duodenal secretion was collected by means of a funnel into a graduated centrifuge tube. Results of 32 experiments, compared with control experiments using 50-100 cc. saline, show clearly that the duodenal glands respond to all three types of 'pure' meals by increasing their rate of secretion. A slight difference in the responses of the two types of preparations suggests that part of the increased secretion in the 'pouch' type may be dependent on motor activity which causes rubbing together of the



mucosal folds and incidental mechanical stimulation. The conclusion is drawn that there is no single specific absorbed secretagogue controlling Brunner's glands, which strengthens the supposition that the humoral agent described as having this property is a hormone.

*Alkaline phosphatase activity of the developing egg and embryo of the grasshopper, Melanoplus differentialis.*

JOSEPH HALL BODINE AND LAURENCE ROCKWELL FITZGERALD. State Univ. of Iowa, Iowa City.

Alkaline phosphatase activity of eggs and embryos of the grasshopper, *Melanoplus differentialis*, of various ages was determined, using a modified King-Armstrong procedure. The activity is expressed as  $\mu\text{g}$ . phenol liberated in 30 minutes per egg (or per embryo). During the first 10 days of development, there is no detectable phosphatase activity in the grasshopper egg. Between the 10th and 21st days of development the activity increases from 0 to about 11. At this time, diapause sets in, but despite the developmental block, the activity doubles by the 80th day, reaching about 22 at this time. If the diapause is broken, the activity increases during the postdiapause period, reaching a level of about 36 at the time of hatching. There is no detectable activity in the embryo proper until the 8th day of postdiapause development (about 10 days before hatching), and this remains very low (2-3) until the time of hatching, at which time the nymph contains all the activity of the original egg. Experiments in which the eggs were centrifuged show that as the embryo becomes packed into the centrifugal pole of the egg, displacing the extra-embryonic fluid, the activity of the centrifugal portion of the egg decreased, and the activity of the central portion increased, as compared with similar sections of an uncentrifuged egg. Experiments in which the extra-embryonic fluid was separated from the embryo, yolk, shell, etc., show that nearly all the activity of the whole egg is to be found in the extra-embryonic fluid. This indicates that the alkaline phosphatase, like tyrosinase, may be formed by the serosa, is found in the extraembryonic fluid, and is incorporated into the embryo at the time of hatching when the embryo swallows this fluid.

*Electrical stimulation of the central nervous mechanism for vomiting in the cat.* HERBERT L. BORISON (introduced by S. C. WANG). Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.

Vomiting has been elicited in 10 out of 20 cats in which the region of the calamus scriptorius in the brainstem was stimulated faradically with a bipolar needle electrode oriented by means of the Horsley-Clarke stereotaxic instrument. Decerebrate preparations were

used in all experiments since anesthesia severely depresses emesis. The graphic response, as recorded with thoracic and abdominal pneumographs, is characterized by an apneusis with vomiting occurring at the peak of inspiration. The main criterion for the elicitation of vomiting, however, was the expulsion of vomitus consisting of milk curd. Stimulating current thresholds for the vomiting response have been determined such that at a constant frequency of 50 impulses per sec., the threshold of intensity is about 8 volts and at a constant voltage of 12 volts, the frequency threshold is between 33 and 50 impulses per sec. By histological identification of the reactive points, the responsive region for emesis has been localized to the area corresponding to the tractus solitarius and the gustatory nucleus and a small portion of the reticular formation lying ventral to these structures. The vomiting center bears a close topographical relationship to the salivatory center (*J. Neurophysiol.* 6: 195, 1943), the spasmodic respiratory center (*Federation Proc.* 7: 10, 1948), and the inspiratory center (*Am. J. Physiol.* 126: 673, 1939). This illustrates the anatomical proximity of the component parts involved in the expression of a complex physiological pattern.

*Effect of testosterone propionate on the total urinary nitrogen excretion of the rat following burns.* JOHN W. BRAASCH AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

The total urinary nitrogen excretion of adult male and female albino rats was followed after an approximately 33% deep second or third degree thermal burn. Constancy of intake was assured by daily force feeding of 22 cc. of a liquid diet made up of 73% carbohydrate, 18% protein, and 10% fat and yielding 2.9 calories per cc. Each sex was divided into three groups of approximately 6 animals each. Two groups were burned, one receiving daily injections of 2.0 mg. of testosterone propionate in peanut oil and the other just peanut oil. In the case of the males, the administration of the drug was begun at the time of the burn. In the case of the females, it was started 2 days before the burn. In addition, one group of both males and females was just injected daily with 2.0 mg. of testosterone propionate in peanut oil. As was anticipated, the urinary nitrogen of the peanut-oil treated, burned rats was elevated for approximately 6 days after a temporary 2- to 3-day depression following the burn. In those animals treated with testosterone propionate, the level of urinary nitrogen excretion remained approximately at the control value. The effect of testosterone propionate on the nitrogen excretion of normal male and female rats was approximately the same for both sexes and also

equal to the calculated effect in the burned animals for 8 to 10 days after the burn. On days 10 to 12 following the burn, the effect of testosterone propionate is no longer evident whereas in the unburned animals, the effect continues unabated.

*Tissue distribution with time following single intravenous administration of sodium pentothal (sodium ethyl (1-methylbutyl) thiobarbiturate).* L. M. BROOKS, J. L. BOLLMAN, E. V. FLOCK AND J. S. LUNDY. Mayo Foundation and Clinic, Rochester, Minn.

We have made a study, using the method of Jailer and Goldbaum (with slight modifications) and also using radioactive pentothal,  $S^{35}$ , to determine the distribution of pentothal at various times after a single intravenous injection of amounts of the drug which produce deep anesthesia in normal rats. Male white rats weighing approximately 200 gm. were given 40 mg/kg. of pentothal into the saphenous vein. One minute after the start of injection 4 to 5 cc. of blood were withdrawn by direct cardiac puncture, the heart excised to stop circulation, and the other organs excised thereafter as rapidly as possible. Other rats were similarly injected and subsequently killed for plasma and tissue analysis 3, 10, 30, 60 or 120 minutes after injection. From our results it is apparent that pentothal is rapidly distributed throughout the body and that the greatest concentration in each tissue is reached within one minute. There is no evidence of subsequent marked accumulation in any tissue studied or of any special affinity for pentothal by any tissue. The concentration in the major tissues is not markedly different from that of the plasma at any time, that found in the liver and kidneys being somewhat higher than that of the plasma and that of the brain, muscle, lung and intestine being approximately equal but slightly less than that of the plasma. Following the immediate distribution of pentothal there is a subsequent slow decline in the amount found in each tissue, this loss from each tissue being at approximately the same rate.

*Survival time of dogs at a barometric pressure of 30 mm.*

Hg. B. H. BURCH (by invitation) AND F. A. HITCHCOCK. Laboratory of Aviation Physiology, Ohio State University, Columbus.

Two series of unanesthetized dogs were explosively decompressed at a rate of 33,650 mm. Hg/sec. and maintained for varying lengths of time at a terminal pressure of 30 mm. Hg. In the first series the animals were exploded from 700 mm. Hg, (2500 ft.), while in the second series, the explosion was from 180 mm. Hg (35,000 ft.). These animals were maintained on 100%  $O_2$  at this altitude for at least 20 minutes preceding the explosion. The initial alveolar  $pO_2$  was thus essentially

the same in both series. Of the 13 dogs making up the first series, none survived the terminal altitude for 80 seconds or longer. In series 2 (17 dogs) no mortality occurred when the duration at terminal altitude was less than 100 seconds, 50% mortality occurred in 100 seconds, and 100% mortality when exposure to terminal altitude was 180 seconds or longer. Recovery in series 2 was faster and more complete than in series 1. A series of experiments designed to determine the immediate cause of death of animals exposed to pressures of 30 mm. or less have been carried out. In anesthetized animals, the anterior chest wall was replaced by a plastic window and observations made on the heart. Fibrillation occurred in about 80 seconds after explosive decompression. Observations made through plastic windows sutured into the right ventricle showed the presence of bubbles in the right ventricle and in the coronary vessels within 35 seconds after the explosion.

*Reproducibility of values for arterial oxygen saturations under varying conditions in a patient with an intracardiac venous arterial shunt.* H. B. BURCHELL AND E. H. WOOD. Mayo Foundation, Rochester, Minn.

Direct and oximetric studies of arterial oxygen saturation have been carried out at approximately monthly intervals for a period of a year on an adult patient with a congenital cardiac defect of the cyanotic type. During supine rest the arterial oxygen saturation determined by Van Slyke analyses averaged 85.0 % and ranged from 81.4 to 88.7%. During this period the oxygen capacity decreased from 33.6 to 20.9 volumes % as a result of monthly phlebotomies. The arterial oxygen saturation (Van Slyke) during the last 60 seconds of a 5.5-minute period of walking at 1.7 miles per hour averaged 65.5 and ranged from 59.3 to 73.4%. The resting arterial oxygen saturation increased on the average to 94.5% and ranged from 92.5 to 95.9 during breathing 100% oxygen. The evidence obtained by means of cardiac catheterization, carried out on two occasions, indicates a constancy in the venous shunt during these conditions. When walking at 1.7 miles per hour, the arteriovenous difference increased and the fall in arterial oxygen saturation was largely related to decreased oxygen content of the venous blood shunted to the arterial side and not to any increase in the percentage shunt. The studies on this and similar individuals apparently indicate that in patients with intracardiac venous arterial shunts who are well compensated from the circulatory viewpoint, the percentage of venous arterial shunt may be relatively constant and that the result of direct and oximetric arterial oxygen saturation studies during supine rest, standing, walking and breathing 100% oxygen are relatively reproducible.

*Relation between pressure and flow in the perfused frog's leg.* ALAN C. BURTON. Dept. of Biophysics, Univ. of Western Ontario, London, Canada.

Whittaker and Winton, Green *et al.*, Roome, Pappenheimer and Maes, and others have carefully investigated the pressure-flow relations in the limbs of mammals. The distensibility of the vessels, and the anomalous viscosity of blood both complicate the interpretation. By the aid of a micro-flowmeter, modified from that of Bozler, the relation has been studied, especially very low pressures, in the Trendelenburg preparation. Flow and pressure are linearly related, but all flow ceases when the pressure falls below a critical value of about 5 cm. of Ringer's solution. When the pressure is lowered abruptly from a high value to below this critical value, reverse flow occurs, while the vessels empty and force fluid against a pressure gradient. This indicates a 'residual tension' in the walls of the smallest vessels, given by the equation  $T = Pr$ . This tension is not due to tissue pressure, and probably not to elastic or smooth muscle tension. It appears to be an interfacial tension between Ringer's solution and the walls, since it is removed by adding wetting agents (bile salts etc.) to the solution.

*Effect of periadrenal ligation on blood pressure of the dog.*

ROBERT G. CANHAM (by invitation) AND GEORGE E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Unilateral, subtotal periadrenal ligations were performed in 9 dogs and blood pressures were followed for one to five months. Six of these dogs were then subjected to similar ligations of the contralateral adrenal; in addition a one-stage bilateral periadrenal ligation was carried out in another dog and blood pressures were followed as before. Finally, in 6 of the original 10 dogs unilateral renal artery constriction with a Goldblatt clamp and contralateral nephrectomy was performed. Aside from one dog which showed an average rise in pressure of 21 mm. Hg following unilateral ligation and 36 mm. Hg following bilateral ligation, none of the animals developed hypertension. Hypertension, however, was produced later by the Goldblatt method in 4 out of 6 dogs that had previously been subjected to periadrenal ligations.

*$\gamma$ -dichroine, the antimalarial alkaloid of Chang Shan.*

K. K. CHEN. Div. of Pharmacology, Lilly Research Laboratories, Indianapolis, Ind.

$\gamma$ -dichroine is a new alkaloid isolated by Chou (J. Am. Chem. Soc. 70: 1765, 1948) from Chang Shan, *Dichroa febrifuga*. Its Q value in ducklings against *Plasmodium lophurae* was estimated to be about 148; and in canaries against *P. relictum*, 137. Dr. L. H. Schmidt of Christ Hospital, Cincinnati, observed that

$\gamma$ -dichroine in the dose of 0.4-0.8 mg/kg. definitely reduced the parasitemia in rhesus monkeys infected with *P. cynomolgi* (personal communication). The LD<sub>50</sub> in mice was  $10 \pm 0.5$  mg/kg. intravenously, and  $2.74 \pm 0.41$  mg/kg. orally. By repeated administration in mice,  $\gamma$ -dichroine caused hydropic degeneration of the liver in certain animals. Vomiting occurred when  $\gamma$ -dichroine was intravenously injected into pigeons. Diarrhea appeared in rabbits when the alkaloid was similarly administered. In anesthetized dogs,  $\gamma$ -dichroine in the dose of 2.5 mg/kg. by vein induced an increase of duodenal peristaltic activity, but had no effect on respiration and blood pressure.

*Further evidence on conversion of CO to CO<sub>2</sub> by tissues including experiments with radioactive carbon.* ROBERT T. CLARK, JR., J. NEWELL STANNARD AND W. O. FENN. Dept. of Physiology and Vital Economics and Atomic Energy Project, Univ. of Rochester, Rochester, N. Y.

Previous studies from this laboratory have indicated that the metabolism of resting heart and skeletal muscle was increased 2 to 3 times when the air surrounding the tissues was replaced by a mixture of 80% and 20% O<sub>2</sub>. Evidence has been presented that this stimulation of respiration was due to the burning of CO to CO<sub>2</sub> by the tissues. In other tissues such as nerve, stomach, liver, and kidney the metabolism was increased only slightly, and in skin there was some inhibition of the normal respiration. In the present investigation the respirometer experiments were carried out as before with the exception that CO containing radioactive carbon (C<sup>14</sup>O) was used in place of the usual CO in the CO-O<sub>2</sub> mixture. Utilizing this procedure it was possible to measure the CO consumed by the tissues in two ways, 1) by determining the excess respiration in the respirometer and 2) by measuring the radioactivity of C<sup>14</sup>O<sub>2</sub> of the respired gases after absorption in Ba(OH)<sub>2</sub>. Such determinations were made on frog heart, skeletal muscle, skin and nerve. The results show that the amount of CO consumed, as calculated from the radioactivity of the CO<sub>2</sub> collected, agreed with that from the volumeter data, thus proving that the stimulation is primarily due to the burning of CO to CO<sub>2</sub>. Also, the rate of burning by each tissue studied was in the same direction as previous studies have shown. The rate was very high in heart and skeletal muscle, low in nerve, and negligible in skin.

*Quantitative determination of inhibitors of gastric secretion.* CHARLES F. CODE, GEORGE R. LIVERMORE (by invitation), HENRY V. RATEKE (by invitation) AND CHARLES M. BLACKBURN (by invitation). Mayo Foundation and Clinic, Rochester, Minn.

It was shown by Gray, Bradley and Ivy (Am. J.

*Physiol.* 118: 463-476, 1937) that the degree of inhibition of gastric secretion produced by enterogastrone is dependent to a considerable extent on the rate of secretion of gastric juice at the time of testing. This and the differences in size between dogs and stomachs or pouches of dogs have made quantitative comparisons of inhibitor activity somewhat difficult. The following procedure has been developed to overcome some of these difficulties and has been used satisfactorily to test some inhibitors. Dogs with Heidenhain pouches were used. The maximal rate of secretion of hydrochloric acid possible from these pouches in response to histamine given every 10 minutes subcutaneously was determined and checked repeatedly. Depending on the sensitivity desired, the inhibitor was injected intravenously while the pouches were secreting at a fourth of this maximal rate when a high degree of sensitivity was required, or at half of the maximal rate or greater if a less responsive, though somewhat more stable, preparation could be used. Administration of the dose of histamine giving the desired rate of secretion was continued until completion of the assay. As a rule the mean of the inhibition produced during the second and third hours after injection of the test substance was used in the calculation of the results. These may be expressed as percentage inhibition per weight or volume of inhibitor or in terms of some unit established for the particular study.

*Hepatic and peripheral removal rates of the dog for intravenously injected bromsulphalein.* CLARENCE COHN, RACHMIEL LEVINE AND MURIEL KOLINSKY. Dept. of Biochemistry, and Metabolism and Endocrinology, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Bromsulphalein disappeared from the blood of eviscerated-hepatectomized-nephrectomized dogs more than three times as rapidly as Evans Blue which was injected intravenously at the same time. No evidence for excessive loss of BSP through the capillaries could be demonstrated in the eviscerate preparation. It was possible to estimate 'Pm' (maximal transfer capacity of the periphery for bromsulphalein), since it was impossible to 'saturate' extra-hepatic and extra-portal sites of bromsulphalein removal with serum levels as high as 100 mg. %. The rate of removal of intravenously injected BSP, being the sum of both portal and peripheral removal mechanisms, invalidates the use of the dye for measuring either hepatic blood flow or of ('Lm').

*Studies on the in vitro secretion of HCl by the mammalian stomach.* FRED E. COY, JR., (by invitation) AND WARREN S. REHM. Dept. of Physiology, Univ. of Louisville, Louisville, Ky.

In 1930 Delrue (*Arch. int. Physiol.* 33: 196, 1930) reported the *in vitro* secretion of HCl by the frog's stomach. In the present work we attempted to obtain *in vitro* secretion of HCl in the mammalian stomach. A piece of stomach, usually with the muscle layers dissected off, was tied around the end of a glass tube and the preparation then immersed in an appropriate fluid (Ringer's or serum) at 38°C., adjusted to a pH of 7.4 through which 95% and 5% CO<sub>2</sub> was bubbled. Saline (or tap water) was placed in the tube in contact with the mucosa and an oxygen mixture bubbled through this fluid. Twelve preparations of dogs' stomach, 12 of rabbits', 6 of hamsters', and 8 of guinea pigs' were used. The secretory rate was determined by draining the fluid in the tube and replacing with fresh saline, usually at 15-minute intervals. The pH of the saline from the tube was measured with a glass electrode. In only three cases did the pH of the saline fall below 5.0. Because of the possibility of an abnormally high CO<sub>2</sub> content of the tissue when 5% CO<sub>2</sub> was used, experiments were performed on guinea pigs with 100% O<sub>2</sub> and phosphate Ringer's. In 10 out of 12 experiments the pH fell into the 4's every 15 minutes for from two to several hours. In 4 experiments performed at 32°C. (see Davies, *Biochem. J.* 40: xxxv, 1946) a higher secretory rate was obtained. The highest rate was one in which the pH dropped to 3.18 in 15 minutes.

*Variations in a single subject of O<sub>2</sub> intake, acetylene minute volume, O<sub>2</sub> debt and RQ on 12 various exercises designed as tests of maximal circulatory capacity.* THOMAS KIRK CURETON, JR., ALFRED W. HUBBARD (by invitation), LOUIS J. SAKAL (by invitation) AND W. KASTRINOS (by invitation). Univ. of Ill., Chicago.

In 15 tests of net O<sub>2</sub> intake on maximal exercises given to a 115 lb. meso-medial subject, of near average running endurance and 68" tall, the range in net O<sub>2</sub> intake was from 1.620 l/min. to 2.780 l/min., S.D. about the mean  $\pm .1865$  and  $\sigma_M^2 = \pm .1154$ . The highest minute volume was reached on the All-Out Step Test (40/min., 17" bench) but the 7 mi/hr. Treadmill Run (8.6% slope) gave 25.25 l/min. A plot of  $\frac{O_2 \text{ intake}}{.123}$  parallels the acetylene MV fairly closely, plotted against Cal/hr/kg., the rank order correlation being .58 but the true relationship is much better than this due to the curvilinear shape of the graph. The subject tensed up at 10 mi/hr. (8.6%) slope and the MV dropped to 18.76 l/min., a drop of 29.5%. The re-test variations in this work averaged 5.12% in net O<sub>2</sub> intake, although some of the tests were several weeks apart. The O<sub>2</sub> debt varied in 15 determinations on 12 different exercises from 3.38 l. to 10.93

l.; of the variations 1.965 l. and  $M \pm .525$  l. The more obvious causes of variation were 1) subject giving up before being 'All-Out', 2) variable recovery times (2) same exercises restricting ventilation. The 7, 10 and 13.5 mi/hr. All-Out treadmill runs (8.6% slope) caused  $O_2$  debts of 9.54, 9.12 and 10.43 liters. The equation derived to fit the curve of relationship between the difficulty of the exercise in Cal/min. (X axis) and duration of exercise (Y axis) is:  $Y = 16.01e^{-.058x}$  (between 20-60 Cal./min.). Above 60 Cal/min the graph is a straight line parallel to the base axis. The logarithmic plot shows all points practically on a straight line except for two exercises below All-Out capacity, one which obviously obstructed circulation (Burpee-Push-Ups) and one in which the subject stopped before exhaustion. The normal sitting RQ of .80 rose to 1.12 in All-Out exercise,  $+1.51$  in the first bag of recovery gas and then dropped back to .80 or below. After three tests it went as low as .67 and after the 13.6 mi/hr. Treadmill Run it dropped to .56 at the end of training. The RQ is systematically lowered by training, the normal resting RQ dropping to .66. Several very highly trained endurance athletes have been found with RQ's as low.

*Effects of microwave diathermy on the eye.* L. DAILY, JR., K. G. WAKIM, J. F. HERRICK AND E. M. PARK-HILL. Mayo Clinic and Foundation, Rochester, Minn.

A study was made of the changes in temperatures of the orbital tissues, aqueous, and vitreous humors and of the pathological changes resulting from exposure of the eye of the dog to microwaves. One series of acute experiments was performed on 36 dogs anesthetized with pentobarbital sodium. Thermocouple needles were used for recording temperatures of the orbit, aqueous and vitreous humors of the eye before and after exposure to microwaves. Exposure of the eye to microwaves for 30 minutes using 75% of the output with the corner director at a distance of 3 inches produced an average temperature rise of 1.9°C. in the orbit, 3.2°C. in the vitreous and 2.8° in the aqueous, exactly one minute after the microwaves were turned off. Another series of experiments was performed on 8 dogs similarly anesthetized and one eye was exposed to microwaves. Both eyes were studied clinically, including ophthalmoscopic examination of the media and fundi before and after exposure to microwaves. Eyes of 2 dogs that were exposed once daily, six and ten times respectively, to microwaves using 75% of the output with the director at a distance of 3 inches for 30 minutes, so far have shown no clinically observable pathologic findings. Eight 30-minute exposures of the eye of one dog once daily using 75% output with the director

at 2 inches produced anterior cortical cataract within six days after the last exposure. Seven similar exposures of the eye of another dog using 98% output with the director at 1 inch produced anterior cortical cataract within 24 hours after the last exposure. Over a period of 9 weeks these anterior cortical cataracts increased in size and density and then regressed and posterior cortical cataracts developed. In one dog, one 30-minute exposure of the eye to microwaves using 75% output with the director at 2 inches produced corneal clouding and partial iridoplegia within 24 hours after exposure. Microscopic examination of sections of this eye revealed round cell infiltration of the corneal stroma. Similar clinical and histologic changes were produced in the eye of another dog within 24 hours after two 30-minute exposures to microwaves, and in addition, vitreous opacities, reddening of the optic disc, whitening and elevation of the retina in the region the disc were observed ophthalmoscopically. Microscopic examination revealed red blood cells, leukocytes and fibrinous exudate in the anterior chamber with hemorrhage into the iris and ciliary processes. Necrosis of iris stroma and disorganization of the pigment layer of the iris and subchoroidal exudate and cystic degeneration of the retina were also observed.

*Metabolism of the mouse stomach in vitro.* HORACE W. DAVENPORT. Dept. of Physiology, Univ. of Utah, Salt Lake City.

The accumulation of acid in the lumen of the mouse stomach has been studied *in vitro*. It has been shown that carbonic, lactic and pyruvic acids account for only a small part of the total acid found, and the remaining acid is believed to be inorganic acid secreted by the parietal cells. Arsenite (0.34 mM) moderately inhibits acid secretion, but the amount of pyruvic acid accumulating in the presence of arsenite is one fourth the deficit in acid secretion. Addition of acetate does not reverse the arsenite inhibition. The total amount of pyruvic acid reduced and oxidized by the stomach is one fourth the amount of inorganic acid secreted. The use of pyruvic acid by the stomach is inhibited by 0.34 mM arsenite, but it is unaffected by 30 mg. % thiocyanate, a more potent inhibitor of acid secretion. Shay *et al.* (*Gastroenterology*, 6: 199, 1946) have shown that severe thiamin deficiency does not reduce total acid secretion in the rat. It is therefore tentatively concluded either that the oxidation of one mole of pyruvic acid must produce four moles of hydrochloric acid or that pyruvic acid metabolism is not an essential part of the acid secreting mechanism.

*Validity and reliability measurements of inulin diodrast function tests.* DEAN F. DAVIES (introduced by HENRY A. SCHROEDER). Section on Cardiovascular

Disease and Gerontology, National Institutes of Health, USPH, Baltimore City Hospitals, Baltimore, Md.

The validity of diodrast Tm was tested at different tubular loads by titration experiments and by repeated tests. In titration experiments 10 of 12 subjects showed a depression of Tm values when tubular load/Tm ratios were increased to an average of 4.81 after control periods during which tubular load/Tm ratios averaged 2.57. In tests repeated on different days the mean Tm values were significantly depressed when plasma diodrast levels were 20 per cent or more above control levels. An examination of reliability of these tests was made by determining reliability coefficients of single periods and of single tests of each function. An attempt was made to differentiate between deviations due to physiological variation and instrumental error by statistical means. It was found that instrumental error plays a negligible role in day-to-day variation. Perfect reliability would decrease the standard error of estimate of inulin clearance from 13.8 to 12.2 cc. plasma per minute, of diodrast clearance from 101.9 to 98.2 cc. plasma per minute, and of diodrast Tm by only 0.4 mg. diodrast iodine per minute. Validity of surface area correction was studied by a preliminary analysis of 37 adult male subjects between 29 and 89 years of age and surface areas ranging between 1.39 and 2.06 square meters. It showed that the correlation between surface area and diodrast clearances and between surface area and diodrast Tm is insignificant. On the other hand, the same individuals showed a significant correlation between basal metabolic rate and each of these functions.

*Cochlear microphonics and action potentials in the guinea pig.* HALLOWELL DAVIS, S. RIESCO-McCLURE (by invitation) AND D. MCAULIFFE (by invitation). Central Institute for the Deaf, St. Louis, Mo.

Electric responses of guinea pigs' cochleas were studied with a three-channel oscilloscope. Electrodes were placed on round window and in small holes (less than 0.1 mm.) opening into scalae vestibuli, media or tympani in turns 1, 2 or 3. Escape of fluid was often avoided; when it occurred it usually initiated slow progressive failure of microphonics and action potentials. At all positions the action potential following a click was nearly identical. At a given position scalae media vestibuli gave the same microphonic responses. Scala tympani gave the same microphonic but reversed in polarity. The latency of each response was also the same (within 0.03 msec.) at all positions. The pick-up of microphonics seemed fairly localized because 1) input-output curves for pure tones differed with position in respect to location on sound-intensity

scale, maximum voltage, presence of 'overload,' and slope and shape of curve; 2) at high intensities the first *sub-harmonic* of frequencies above 3500 might be equal to the fundamental at turns 2 and 3 although barely detected at round window; and 3) interference effects between two tones might be quite different at turn 2 and round window. Nevertheless, the response at one position did *not* show any marked maximum or special sensitivity relative to frequency, provided the input was adjusted to keep the response at another position constant. There was merely a gradual increase in low-tone emphasis as the electrode approached the apex. With constant sound intensity there was a strong maximum (natural period) at 1700 cps.

*Estimation of pulmonary capillary pressure.* L. DEXTER, F. W. HAYNES, AND H. K. HELLEMS. Medical Clinic, Peter Bent Brigham Hospital and Dept. of Medicine, Harvard Medical School, Boston, Mass.

A cardiac catheter with the hole on the tip was introduced into a small branch of the pulmonary artery of dogs so as to obstruct the arterial lumen. Another catheter was introduced through the arterial system into the pulmonary vein so as to obstruct the venous lumen. Pressures were recorded with Hamilton and saline manometers. The pressure existing in the lumen of the artery distal to the obstruction is a result of the retrograde transmission of pressure from the next collateral branch entering the pulmonary artery. Anatomically, this is the pulmonary capillary bed. Physiologically, this is also the case since blood fully saturated with oxygen can be withdrawn through the catheter occluding the pulmonary artery. Pressures in the catheter obstructing the pulmonary vein average 4 to 6 mm. Hg higher than in that obstructing the pulmonary artery. The same held true when both pressures were raised by producing pulmonary embolism with lycopodium spores. When respiration was halted, there was little change in the relationship of the pressures, but when blood flow ceased (death), the pressures became equal indicating that the differences in the two pressures were largely a function of blood flow. It is concluded that the pressure recorded through the obstructed artery was less because blood flow was reduced locally and that through the obstructed vein greater due to passive congestion. Averaging of both pressures gives a means of estimating the absolute magnitude of pulmonary capillary pressure. Values obtained lay between 8 and 9 mm. Hg.

*Man's ceiling as determined in the altitude chamber.* D. B. DILL AND K. E. PENROD. Medical Div., Army Chemical Center, Md.  
Experiments were carried out in the Aero-Medical

Laboratory, Wright Field, early in the war on how high man can fly breathing oxygen. This paper reports observations at 44,800 feet, the highest altitude maintained for any length of time without pressurization. The eight men exposed to this altitude from 15 to 44 minutes, exhibited a variety of respiratory adjustments. Pulmonary minute ventilation ranged from 15 to 56 l., arterial oxygen saturation, from 58 to 84% and arterial  $pH$  from 7.45 to 7.76. No one lost consciousness and only one became slightly tetanic. One of the 8 experienced after effects attributable to anoxia, headache, nausea and visual disturbances. All were in a state of imminent collapse. The calculated pressure gradient of oxygen from alveoli to arterial blood was zero in those with minimal alveolar ventilation but was 16 mm. Hg in the man with maximal ventilation (alveolar  $pCO_2$  12 mm. Hg). In men with alveolar  $pCO_2$  between 30 and 24 the  $\Delta pO_2$  ranged from 6 to 13. The failure to reach oxygen equilibrium in hyper-ventilation may reflect a reduction in time available for oxygen transfer, i.e., to faster blood flow through the pulmonary capillaries.

*Studies of pulmonary function with the use of the oximeter.*

J. C. DOUGLAS AND O. G. EDHOLM. Univ. of Western Ontario, London, Canada.

Blood in the left heart or systemic arteries contains fractions from well and poorly ventilated portions of lung which have come into equilibrium with each other. Therefore, under a given set of conditions, the mixed arterial saturation may be expected to reflect the adequacy of pulmonary function. Using the Millikan Oximeter as an indicator of arterial oxygen saturation, a study has been made of the increase in  $pO_2$  of the inspired gas required to raise saturation to a given value (i.e. 100%) from its level of 95-98% when breathing room air. In normal subjects, an alveolar oxygen tension of  $227.8 \pm 13.8$  mm. Hg produces complete saturation. This corresponds roughly to an oxygen concentration of 40%. The time required to reach complete saturation when the subject changes from breathing room air to 100% oxygen has also been determined—termed 'saturation time'. This, expressed as half-time, has been found to be  $17.0 \pm 1.7$  seconds in normal subjects. In subjects with varying degrees of pulmonary damage, caused by a number of diseases, significant differences in respect to both these measurements have been found. The results obtained so far correlate satisfactorily with independent clinical estimates of the subject's pulmonary disability. To find a known value at which the oximeter could be set with confidence, in every case blood-gas analysis was performed on a sample of arterialized-venous blood, taken while the subject was breathing pure oxygen, to

ascertain that 100% oxygen produced complete saturation.

*Tissue stimulators utilizing radio frequency coupling.*

DONALD R. DUBBERT (introduced by OTTO H. SCHMITT). Univ. of Minnesota, Minneapolis.

The problem of isolating voltage stimuli used in tissue studies both conductively and capacitively from ground is discussed. A relatively simple method of achieving this isolation by means of radio frequency coupling is described in detail. The required stimulating voltage is used to amplitude modulate a radio frequency oscillator which is inductively coupled to a receiving circuit where the modulated carrier is reconverted into the original voltage stimulus by means of a small germanium crystal diode, operating as a linear rectifier, followed by a radio frequency filter. The resulting isolation is conductively complete, and the capacitance from output is of the order of only a few micro-microfarads. Certain design details which are of importance in obtaining satisfactory operation of the radio frequency stimulator as well as performance data are presented.

*Effects of an antihistaminic in experimental shock.*

H. E. EDERSTROM, M. R. DIGANGI AND CARL CALMAN (by invitation). Dept. of Physiology, St. Louis Univ., St. Louis, Mo.

Rats anesthetized with sodium pentobarbital were shocked by application of rubber bands to the hind legs for 3 hours and 40 minutes. Before release of the tourniquets 62 rats were given 20 mg/kg. Benadryl intraperitoneally. The 24-hour survival rate was 10% for the treated group, and 24% for 83 untreated controls. In dogs anesthetized with morphine and sodium pentobarbital burn shock was induced by immersion of hind limbs and lower abdomen in water at 90°C. for 5 seconds. Before immersion 16 animals were given i. m. 20 mg. Benadryl/kg, followed by 5 mg/kg. every 3 hours after the burn. The 24-hour survival rate in the treated group was 19%, and 25% in 16 untreated dogs. A second series of shocked dogs was treated initially with Benadryl as described above, but after the burn 40 mg. % Benadryl in saline was administered by intravenous drip at a rate permitting 100 cc/kg. to flow per 24 hours. Control animals received saline only at the same rate. In 11 Benadryl-treated animals survival was 36%, and in 11 controls was 27%. Blood pressure readings taken at hourly intervals in shocked dogs showed no significant differences between control and treated animals. Hematocrit readings taken every 3 hours suggested that the Benadryl-treated dogs did not show as great hemoconcentration as the controls.



*Oximetry respiratory patterns in arterial hypoxemia with and without intra-cardiac shunt.* JAMES O. ELAM, ALBERT ROOS AND J. F. NEVILLE, JR. (introduced by H. L. WHITE). Laboratory of Applied Thoracic Physiology (Surgery) and Dept. of Physiology, Washington Univ., St. Louis, Mo.

Fluctuations in the oximeter value as a result of the respiratory cycle have been studied in two groups of hypoxemic patients, the first group having no evidence of intra-cardiac shunt and the second group having interventricular septal defects demonstrable by cardiac catheterization studies. In the first group of hypoxemic patients the respiratory pattern in the oximeter value consisted of an increase in saturation 5-7 seconds after the beginning of inspiration followed by a decrease in saturation 5-7 seconds after the beginning of expiration. Such patterns were observed when the patient breathed room air at a rate of 4-6/min. and disappeared on breathing 100% oxygen. Apparently the slow respiratory rate permits depletion of increased alveolar  $pO_2$  during expiration so that the following inspiration elevates the alveolar  $pO_2$  and hence the relatively unsaturated arterial blood. The 5 to 7-sec. interval between the beginning of inspiration and the increase in the saturation agrees with the accepted values for lung-to-ear circulation time. The second group of hypoxemic patients who had intra-cardiac shunt showed a different respiratory pattern in the oximeter values with slow deep breathing. In this group the saturation decreased 2-3 seconds after the beginning of inspiration and increased 2-3 seconds after the beginning of expiration. Such patterns were elicited while the patient breathed room air or 100% oxygen. It appears that expiration caused a decrease in flow of the venous shunt blood relative to the oxygenated blood entering the aorta and that inspiration reverses this effect so that relatively more of the shunt blood enters the aorta. The 2 to 3-sec. interval between inspiration and the saturation increase suggests heart-to-ear circulation time. Occurrence of the pattern during breathing of 100% oxygen excludes the alveolar  $pO_2$  role in this pattern as distinguished from the respiratory pattern obtained in patients without intra-cardiac shunt.

*Cross-acclimatization to heat and cold.* JOHAN W. ELIOT, HAROLD J. STEIN AND RICHARD A. BADER (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass.

Three healthy men were physically conditioned by walking 7 miles a day for 10 days, and were then exposed successively to 19 5-hour periods of heat (107°F.

dry bulb; 89°F. wet bulb; and 3 m.p.h. wind velocity), 14 5-hour periods of cold (-20°F., wind 3-4 m.p.h.), and 5 re-exposures to heat. After 5 weeks without environmental stress, 3 more heat exposures were given. The most striking finding of these studies was that, in men acclimatized to heat, loss of acclimatization was not accelerated by repeated cold exposures, as judged by cardiovascular and metabolic indices and subjective reactions. More rapid and effective vasoconstriction with successive cold exposures was demonstrated. Continuous diuresis and increased chloride loss were observed during the period of cold exposures, and these trends were only slowly reversed upon re-exposure to heat. No significant difference in basal metabolic rate was observed between the hot and cold periods. Blood, plasma and 'available fluid' volume studies revealed no consistent changes during the hot or cold periods, but plasma protein and hematocrit values showed definite hemoconcentration in the cold and hemodilution in the heat. It has not been demonstrated that increased tolerance to cold results from any of the changes observed.

*Single fiber limulus nerve preparation for action potential analysis.* LESTER ERICKSON (introduced by Otto H. SCHMITT). Univ. of Minnesota, Minneapolis.

Limulus leg nerves were studied as a possible source of single nerve fibers for dissection by the technique of Hodgkin. Histological examination showed the presence of numerous axons larger than 20 microns in a circumscribed area of the nerves studied. Histological data on these nerves was not found in the literature so fiber distribution studies were made similar to work done on squid by Young. Enlarged photomicrograph prints were used for these studies. The total number of fibers in Limulus leg nerves was estimated to be about 8000. Detailed fiber measurement and counts from the circumscribed area of the nerve containing the large axons gave an average of 435 fibers in the area. In the circumscribed area, 51% of the fibers were smaller than 5 microns; 21% of the fibers were from 5-10 microns; 18% were from 10-15 microns; 5% were from 15-20 microns in diameter and 5% were greater than 20 microns in diameter. When histological study showed that Limulus nerves might be a satisfactory source for single fiber preparations, dissection of the nerves was attempted. It was found possible to isolate the large diameter axons of 20 microns and make satisfactory single fiber preparations from such fibers. The technique and equipment was perfected so that preparations could be made for oscillographic analysis of action potentials propagated by single fibers.



*Effect of vagotomy on intestinal motility.* S. FAIK, F. C. MANN, AND J. H. GRINDLAY. Mayo Foundation, Rochester, Minn.

A study was made of intestinal activity, photographically recorded, in a group of trained dogs before and after transthoracic vagotomy. The apparatus was designed to record the slightest change of diameter in the intestine, and was attached to exteriorized loops prepared at different levels of the alimentary tract. In several instances 3 loops were prepared on the same dog, duodenal, jejunal and ileal. Various stimuli were applied and recordings were made. Emptying time of the stomach was delayed after vagotomy. This was shown by the necessity of fasting the dog for 48 hours in order to show complete quiescence in the loops in contrast to 26 hours fast in the nonvagotomized dog. Vagotomy abolished the intestinal activity caused by the slight and smell of food. The feeding reflex was delayed and of a shorter duration. Peristaltic waves were less in number, of shorter duration and occurred at longer intervals. This was especially marked in the ileum. The rhythmic waves were not affected in number or in their law of gradience.

*Effect of diabetes and insulin on glucose Tm and other renal functions.* SAUL J. FARBER, NEAL J. CONAN, JR., AND DAVID P. EARLE, JR. (introduced by HOMER W. SMITH). New York Univ., New York City.

The maximum tubular reabsorption of glucose (TmG) by the kidney was examined in 10 diabetics. Filtration rates were found to be within normal limits as were most of the absolute TmG values. However the GF/TmG ratios were lower than normal indicating a disproportion in glomerular and tubular function. Thus, the kidney tubule of the diabetic reabsorbs more glucose in proportion to the amount of glucose filtered. The action of insulin on the glucose Tm was examined in normals and diabetics. Insulin reduced the Tm in some of the normals examined and in all the diabetics. The GF/TmG ratio increased toward normal value in the diabetics in approximately the same proportion as the decrease in TmG. In several patients the excretion of glucose actually increased after insulin despite the fall in blood sugar. The effect of glucose and glucose plus insulin on the excretion of Na, K and  $\text{PO}_4$  was examined in a limited number of diabetics. The excretion of K and  $\text{PO}_4$  usually decreased as the blood level fell after the administration of glucose and glucose plus insulin. The excretion of Na increased after glucose. In a few instances the excretion of Na diminished after insulin despite the fact the excretion of glucose increased.

*Secretion of insulin in the dog studied by means of the pancreatic-femoral anastomosis.* PIERO P. FOA, JAY A. SMITH (by invitation), AND HARRIET R. WEINSTEIN (by invitation). Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

A disagreement exists regarding the nature of the hypoglycemic phase of the normal glucose tolerance curve. Some authors believe that it is due to stimulation of insulin production, while others attribute it to a protracted inhibition of liver glycogenolysis. The problem was investigated by means of 38 cross-circulation experiments on fasted heparinized dogs. The pancreato-duodenal or a branch of the mesenteric vein of the donor (A) was anastomosed with the femoral vein of the recipient (B). Return circulation was from the femoral artery of B to the femoral vein of A. After a control period, 5 cc/kg. of a 20% solution of glucose or the same volume of iso-osmotic (4%) saline was injected intravenously into A. Blood samples were taken for 75 minutes following the injection, the anastomoses were then disconnected and the sampling continued for 2 more hours. Blood sugar was determined according to Folin and Malmrose. The glycemia of a dog receiving pancreatic blood from a donor injected with glucose decreases sharply reaching a minimum in 30 to 60 minutes and gradually returning toward normal after the anastomosis has been disconnected. No changes are produced if saline was injected into a dog A. When the mesenteric vein of A was used, the glycemia of B increased instead of decreased. The hypoglycemia of B was of the same order of magnitude as that observed in the normal glucose tolerance curve, and appeared to be due to an increase of insulin content in the pancreatic blood of A. The results are consistent with the hypothesis that a rise in blood sugar concentration stimulates the secretion of insulin and that the pancreas plays a primary role in the production of the hypoglycemic phase of the normal glucose tolerance curve.

*A rapid infrared gas analyzer.* RICHARD C. FOWLER (introduced by W. O. FENN) Dept. of Physiology and Vital Economics, Univ. of Rochester, Rochester, N. Y.

A selective gas analyzer, suitable for operation with any gas having a unique infrared absorption band of measurable magnitude is described. These include  $\text{CO}_2$ , CO, ether, acetylene, nitrous oxide, and others. Its features at present include an instantaneous sample volume of approximately 0.5 cc. obtained by crossing the measuring beams, and an overall response time of 90% full scale in 0.15 seconds obtained through the use of rapid 'SETT' bolometers. It is

adaptable for both static and continuous flow measurements. A calibration curve and sample results are shown.

*Non-uniformity of lung ventilation.* WARD S. FOWLER (introduced by JULIUS H. COMROE, JR.). Dept. of Physiology and Pharmacology, Univ. of Pennsylvania, Philadelphia.

Continuous analysis (Lilly-Hervey nitrogen meter) of  $N_2$  content and volume flow of alveolar gas expired after one inspiration of 99.6%  $O_2$  showed the  $N_2$  content to increase several per cent as expiration continued. This indicates that a) inspired gas is not evenly distributed throughout the functional residual air and b) with quiet breathing the poorly ventilated areas of the lung empty proportionately more later in expiration. In 38 of 40 healthy men, increasing  $N_2$  content was found in alveolar gas of a quiet tidal expiration. The magnitude of the variation in  $N_2$  content has been expressed in terms of the relative dilution of alveolar  $N_2$  by inspired  $O_2$ , and was affected by inspired volume, pre-inspiratory lung volume, inspiratory breathholding, expired volume and manner of expiration. When inspiration began at the maximal expiratory level, added dead space appeared to increase the  $N_2$  variation. Decreased variability of  $N_2$  content was found with voluntary and post-exercise hyperpnea.

*Action potentials from single auditory nerve fibers.*

ROBERT GALAMBOS AND HALLOWELL DAVIS. Harvard University, Cambridge, Mass., and Central Institute for the Deaf, St. Louis, Mo.

We have previously reported on the electrical response of single units in the auditory nerve of cats: When a glass micropipette is introduced into the 'nerve', sharp spikes are elicited by acoustic stimuli. These spikes have been assumed to represent conducted impulses in auditory nerve fibers. For various reasons, it now seems more likely that they represent the discharge of the soma of second order cell bodies in the auditory tract. Some anatomical and physiological reasons for this conclusion will be presented.

*Cortical projection of proprioception.* J. R. GAY AND E. GELLHORN. Laboratory of Neurophysiology, Univ. of Minn., Minneapolis.

Little direct evidence has appeared in the literature on the effect of proprioceptive impulses in the cerebral cortex in animals and man. The proprioceptors were stimulated by passive movements of the extremities or stimulation of the peripheral end of ventral spinal nerve roots by condensor discharges. Recordings were made with an ink-writing crytograph from the exposed cerebral cortex in 36 cats and 8 monkeys under Dial-urethane anesthesia. It was

found that stimulation of the proprioceptors by passive movements of the extremities or stimulation of the peripheral end of a ventral spinal root resulted in an excitation of the electrical potentials of the contralateral sensorimotor area in the cat and the contralateral precentral motor area in the monkey. The excitation tended to be more diffusely spread in the contralateral hemisphere in the cat. In the monkey the excitation was largely restricted to the contralateral precentral motor cortex. These experiments support the work of Bard and his associates and indicate that the cerebral cortex is involved in the proprioceptive system. It would appear that the cortical projection area of proprioceptive impulses is the sensorimotor area in the cat and the precentral motor cortex in the monkey.

*Proprioception and the motor cortex.* E. GELLHORN. Laboratory of Neurophysiology, University of Minn., Minneapolis.

The effect of proprioceptive impulses originating in muscles under conditions of temporary fixation of one or more joints was studied on the reactivity of the motor cortex to electric stimulation by means of EMG's. Results:

Fixation of a muscle at increased length (e.g., fixation of the elbow at an obtuse angle for the biceps) greatly increases the response of this muscle to stimulation if the proper cortical focus was chosen. This effect is largely due to impulses set up while the muscle develops tension isometrically. Proprioceptive impulses thus induced become also effective in those muscles with which the proprioceptively excited muscle forms specific functional associations. For instance, proprioception originating in the biceps increases also the reactivity of the extensor carpi to cortical stimulation; similar relations exist between triceps and flexor carpi, triceps and hamstrings, and hamstrings and anterior tibial muscle. It was shown also that this effect occurs in the opposite direction within these complexes. For example, fixation of the wrist in ventroflexion increases the reactivity to cortical stimulation not only of the extensor carpi but also of the biceps muscle. These effects seem to be based on the interaction of proprioceptive impulses with those elicited by cortical stimulation, resulting in an increased number of discharging motor units. This interpretation is suggested by the decreased summation time, the greater and more rapidly increasing amplitude of the EMGs and occasionally, by the increased after-discharge.

*The membrane potential of single muscle fibers.* R. W. GERARD AND G. LING (by invitation). Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

We have previously reported (*Federation Proc.*, 1948, and in press) that all surface fibers of the frog's sartorius normally have a membrane potential (measured with a capillary microelectrode) within a couple of millivolts of 80. This value is insensitive to changes in pH, calcium, phosphate, or length, but varies with potassium, stimulation and disturbed metabolism. An A fraction, above 55 m<sup>B</sup>, is directly dependent on metabolism (CrP concentration); a B fraction, below 55 m<sup>B</sup>, is only indirectly so. With both glycolysis and respiration blocked (IAA ± CN), the potential falls to zero in 2 to 4 hours at room temperature. Two points are emphasized here. 1) Muscle fibers may be in full rigor (isotonic CaCl<sub>2</sub>) yet exhibit a normal membrane potential. Conversely, fibers may have a potential under 20% normal (caffeine, high KCl and CaCl<sub>2</sub>) and yet give seemingly normal contractions. There is no necessary parallelism between relaxation, contraction, or contracture and the size of the membrane potential. 2) Stretch does not alter the membrane potential, but is known to increase the resting metabolism (Feng). Since a muscle fiber may be normal at various lengths and since its surface area must vary as length  $\frac{1}{2}$ , it seemed possible that the extra metabolism of stretched muscle was required to maintain the extra area of membrane fully polarized. If so, when metabolic energy is blocked (IAA + CN), a stretched muscle should depolarize faster than its unstretched partner. This is the case. Preliminary results indicate that the rate of depolarization may be proportional to total fiber surface; which would suggest that much of the resting metabolism is used to maintain membrane integrity and polarization.

*Effect of microwave diathermy on the peripheral circulation and on tissue temperature in man.* J. W. GERSTEN,

K. G. WAKIM, J. F. HERRICK AND F. H. KRUSEN.  
Mayo Clinic and Foundation, Rochester, Minn.

The effect of microwaves at a frequency of 2450 megacycles/sec. on the peripheral circulation and on the tissue temperature was studied in 50 normal subjects. Two hundred fifty four observations were made, with the output of the microwave generator either 60 or 80 watts, and with the duration varying from 1 to 30 minutes. The hemispherical director with a diameter of 9.5 cm. was used at a distance of 5 cm. from the exposed surface. The blood flow in the forearms was determined by the use of the plethysmograph with the compensating spirometer recorder, and the skin, subcutaneous and muscle temperatures were recorded by means of thermocouples. After control blood flow and temperatures were established the forearm was exposed to microwaves, and blood flow and

temperature studies were again made when the microwaves were turned off. Significant increases in blood flow and in tissue temperature were produced in the extremity exposed to microwaves, but the changes in body temperature, heart rate, and blood flow of the unexposed extremity were insignificant. The average rise in muscle temperature was significantly greater than that of subcutaneous tissue, while the average increase in subcutaneous temperature was greater than that of the skin. The average increases in temperature were highest after 20 minutes of irradiation, and were practically the same for 60 and 80 watts. After 20 minutes irradiation with 80-watt output, the temperature rise was 6.7°C. in the muscle, 5.8°C. in the subcutaneous tissue, and 4.7°C. cutaneous. With heating prolonged to 30 minutes, the tissue temperature decreased significantly from the level reached at 20 minutes, with a greater decrease after 80-watt than after 60-watt output. The average blood flow increase in the exposed extremity after 30 minutes of heating was 65%. An S-shaped curve resulted when blood flow increase after exposure at 80 watts was plotted against duration of exposure. After 30 minutes of heating the decline in tissue temperature from the peak attained at 20 minutes was proportional to the increase in blood flow.

*Changes in response and in excitability of the perfused turtle ventricle with 'fatigue' and with strophanthin.*

A. S. GILSON, JR. Dept. of Physiology, Washington Univ., St. Louis, Mo.

Experiments have been conducted with the turtle ventricle using a Straub cannula and ligation between atria and ventricle. With bicarbonate buffered oxygenated solutions, addition of strophanthin gave no improvement of mechanical response. Inconstantly, the drug caused prolongation of the measured relatively refractory period but this occurred during a brief period of increasing threshold leading to frank intraventricular blocks. Phosphate buffered solutions yielded results in this latter respect of generally similar nature. A few preparations perfused with phosphate buffered solution for times approaching 30 hours were driven at rates approximately 30 per minute except for brief periods of testing. Driving at more rapid rates, early in the experiment, shortened the R-T interval progressively to a value slightly below normal for the rate used and there was slight shortening of R-T below normal, though only for a few beats, upon return to slower driving rate. With progressive mechanical failure there was progressive shortening of R-T (and of mechanical systole) the change being slight for short cycle lengths, great for long cycles. It was not possible to measure consistent increases of relative refractory

phase adequate to account for this on a basis of the assumption that shortening of the duration of systole measures relative refractoriness. Thus, in the development of the hypodynamic state by this means, minimum duration of systole is but slightly shortened, maximum duration is greatly shortened. This condition is apparently not necessarily or directly related to changes in the duration of the measured relative refractory phase.

*Olfactory acuity and the sensation complex of appetite and satiety: The influence upon olfactory acuity of defatted, dehydrated duodenum of hog (viobin).* MARGARET GOLDSCHMIDT (by invitation), PHILLIP J. RAIMONDI (by invitation) AND FRANZ R. GOETZL. Dept. of Medical Research, Permanente Foundation, Oakland, Calif.

Olfactory thresholds (Elsberg's method) were determined in normal individuals daily at regular intervals. On test days the subjects' statements regarding appetite and their caloric intake were recorded. Meals were found to be preceded by a period of increasing and followed by one of decreasing olfactory acuity. The precibal increase in olfactory acuity could be prevented by intercibal ingestion of food, the decrease in that acuity by omission of meals. It is suggested that by estimating diurnal variations in olfactory acuity, measures may be found for the sensations of appetite and satiety. Benzedrine was observed to produce decrease in olfactory acuity, decrease in the intensity of the sensation of appetite, decrease in caloric value of freely selected meals and, also, a sensation of satiety. It was felt that the conversion of the sensation of appetite into one of satiety as normally follows ingestion of food may depend upon results of interaction between certain constituents of food with certain components of the gastro-intestinal tract. Such interaction might lead to formation or release of substances directly responsible for the change in sensation. Thinking along these lines, it appeared interesting to investigate possible influences upon the sensation complex of appetite and satiety, upon olfactory acuity, and upon caloric value of freely selected meals of defatted, dehydrated but otherwise unaltered duodenum of hog (VioBin). In the experiments presently reported, it was found that in normal human subjects defatted, dehydrated duodenum of hog upon oral ingestion is capable of decreasing olfactory acuity, of depressing the sensation of appetite, of creating a sensation of satiety and probably also of diminishing food intake. Structure and occurrence in other tissues of the constituent of the duodenal preparation responsible for the effects described are being subjected to further investigation.

*Myocardial lactate and pyruvate metabolism in normal intact dogs, as studied by coronary sinus catheterization.* WALTER T. GOODALE, DONALD B. HACKEL, MARTIN LUBIN AND PAULINE P. WILSON. Medical Div., Army Chemical Center, Md.

Myocardial utilization of lactate and pyruvate has been demonstrated by several investigators in isolated heart, heart-lung, and open chest experiments. Development in this laboratory of a technique of catheterizing the coronary sinus under fluoroscopic control has made it possible to study myocardial metabolism in normal intact dogs, unanesthetized as well as under light nembutal anesthesia. Measuring coronary A-V differences, and coronary blood flows by the nitrous oxide method, lactate and pyruvate utilization was found to be even greater than in less physiological preparations. The total lactate and pyruvate uptake accounted for 20-70% of the total myocardial uptake of oxygen. Of greatest interest was the similar linear relationship between the utilization of these two metabolites and their arterial concentrations, whereas no such relationship was found for glucose. Coronary A-V glucose differences were usually low, with such a high sampling error as to be statistically insignificant. Lactate and pyruvate appear to be preferred sources of energy for the myocardium, and are increasingly utilized as their arterial levels rise. This fact may provide a logical explanation for the excellent adaptation of the heart to stress, as to the increased work of exercise, during which lactate and pyruvate tend to accumulate in the blood from contraction of the skeletal muscles.

*Influence of emotions and feeling states on the behavior of the human colon.* WILLIAM J. GRACE, STEWART G. WOLF AND HAROLD G. WOLFF. With the technical assistance of CATHERINE R. LEE AND PAUL SETON. Depts. of Medicine and Psychiatry of the New York Hospital and Cornell Univ. Medical College, New York City.

Previously attempts at studying the function and behavior of the human colon have been made by using x-rays, motor meals, balloon kymography, inspection of the colon through sigmoidoscopy, and inspection of the activity of exposed loops of bowel. The viewpoint of most of the studies has been pharmacologic and descriptive. We have had a unique opportunity to study the behavior of the human colon in two fistulous subjects with particular emphasis of the influence of emotions and feeling states. Our findings indicate that situations productive of anger, guilt, resentment, and hostility are accompanied by hyperfunction of the large bowel. This hyperfunction is manifested by an increase in motor activity, blood flow lysozyme production, and usually an increase in mucus secretion.

In life situation productive of intense fear and fright there occurred a pallor, and relaxation of the large intestine. Increase in motor activity, blood flow and secretion of the large bowel occurred regularly following the ingestion of an average meal. However in one of our subjects little change in activity was noted when he was in a period of low spirits, dejection and mild depression. Other threats to bodily and personal integrity such as sigmoidoscopic examinations, personality study and having the patient perform a psychometric test resulted in an increase in motor activity and blood flow. A period of sustained anger, resentment and hostility resulted in a profuse eruption of petechiae throughout the surface of the colon.

*Effect of bile on gastric mucus.* RHODA GRANT (by invitation), M. I. GROSSMAN AND A. C. IVY. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

The effect of bile on mucus secreted under acute conditions by the cat's gastric mucosa after exposure to 1% acetic acid has been studied. Mucus collected under these conditions is opaque, white, extremely viscous, with a pH of 7-8.5 except occasionally towards the end of the secretion period when it falls to 2 to 3. The change in reaction does not change its gross appearance. Microscopically, however, intact gastric surface cells are suspended in the alkaline; the nuclei alone in the acid state. Bile or bile salts formed with the mucus, at room temperature and at an optimum pH in the range 7 to 8.8, a transparent homogeneous mixture. Its viscosity, in contrast to that of the original mucus, could be measured by the Ostwald method. When mixed with saline, clear acid gastric juice, 0.2M  $\text{Na}_2\text{HPO}_4$  or duodenal pouch juice the mucus remained in a separate phase even in the optimum pH range. The cellular elements disappeared from the mixture. Opacity and high viscosity of the original mucus was due apparently to the cellular elements suspended in it. Disintegrative changes in the gastric surface cells have been found in mucosa exposed to bile for a few minutes—the extent of this depending on the state of maturity of the cells—consequently on the amount of mucus they contained. Cytolysis of these cells by bile suggests potential danger to the mucosa should suitable conditions for the action exist.

*Influence of environmental temperature on the metabolic response to injected pyrogens.* RONALD GRANT (introduced by VICTOR E. HALL). Dept. of Physiology, Stanford Univ., Calif.

Rabbits given typhoid-paratyphoid vaccine (0.01 ml/kg. of vaccine containing  $2 \times 10^9$  organisms per ml.) show a biphasic temperature rise, beginning when

heat defence mechanisms become inhibited, about 15 minutes after injection. Interruption of the temperature rise due to restored activity of heat defence mechanisms occurs in the second hour. Renewed inhibition causes the second rise. At 0°C. the fever curve is similar but with less rise. Shorn animals exposed to 0°C. maintain constant rectal temperatures with strong shivering, oxygen consumption being increased 120 per cent. Given vaccine, they may show slight fever in the first hour but usually develop decided hypothermia in the second hour with obvious reduction of shivering. Fall of temperature may be checked in the third hour by restoration of shivering. Oxygen consumption is slightly increased during the first hour (mean increase 1.76 ml/kg/min., or 17% of the control value at 20°C.). This increase is maximal 20 to 30 minutes after injection. Magnitude of the increase is unaffected by environmental temperatures within the limits of heat and cold tolerance. In the second and subsequent hours at moderate and high temperatures oxygen consumption remains elevated largely because of the van't Hoff effect. At low temperatures there is decided inhibition of oxygen consumption in the second hour, especially in shorn animals, with recovery, usually, in the third hour. Closely similar results were obtained using 'Pyrexin' (an endogenous pyrogen prepared by Menkin from aseptic pleural exudate).

*Aerobic glycolysis in the anesthetized dog.* MELVIN GRAY (by invitation), LOWELL E. HOKIN (by invitation) AND WARREN S. REHM. Dept. of Physiology, Univ. of Louisville, Louisville, Ky.

Lutwak-Mann (*Biochem. J.* 41: 19, 1947) reported aerobic glycolysis in the presence of added glucose in the rat's stomach. The present work is concerned with the rate of glucose consumption and lactate production in the secreting stomach of the anesthetized, heparinized dog. A portion of the stomach with an intact blood supply was placed in a chamber. The vein from the stomach in the chamber was cannulated and the total outflow was collected. Arterial blood was collected simultaneously. The volume of secretion and the quantity of HCl secreted were determined. The glucose consumption and lactate production were calculated from the A-V difference, volume of secretion, and blood flow. The Shaffer-Hartmann-Somogyi method was used for glucose and the microdiffusion method of Winnick for lactate. Glycogen determinations were made on samples of stomach by the method of Good, Kramer, and Somogyi, both before and after the experimental periods. In five out of six dogs the decrease in glycogen was less than 10% of the glucose consumption. The average lactate production was 25% of the glucose consumption. The rate of HCl

production was roughly proportional to glucose consumption. These data disprove the Bull-Gray theory (*Gastroenterology* 4: 175, 1945) of HCl formation because much more HCl is secreted than could originate from glucose going to lactic or pyruvic acid (see Davies *et al.*, *Nature* 159: 468, 1947).

*Gastroduodenal ulceration in dogs produced by continuous intragastric administration of HCl-pepsin solutions.*

M. I. GROSSMAN AND M. J. FOGELMAN (by invitation). Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

In previous experiments we had shown that continuous intragastric infusion of 0.1 N HCl at a rate of 10 cc/kg/hr. for 72 hours produced gastro-duodenal ulceration in all dogs and this effect could be prevented by simultaneous continuous intravenous infusion of isotonic NaHCO<sub>3</sub> at a rate sufficient to maintain normal blood pH. In the present experiments three groups of dogs were used. *Group I*, consisting of five animals, received 0.10 N HCl with 0.2% pepsin intragastrically; *Group II*, 14 dogs, received 0.10 N HCl-pepsin intragastrically plus isotonic NaHCO<sub>3</sub> intravenously in amounts sufficient to maintain normal blood pH; *Group III*, 14 dogs, received 0.15 N HCl-pepsin plus NaHCO<sub>3</sub> intravenously to maintain normal blood pH. *Group I* dogs all developed severe acidosis (blood pH 6.9-7.2) and gastroduodenal ulceration within three days. None of the dogs in *Group II* developed gastroduodenal ulceration even when the experiment was continued up to 10 days. All dogs in *Group III* which were kept going for 6 days or longer developed gastroduodenal ulcers (7 of 14 dogs). Under the circumstances of these experiments the threshold of acid concentration for ulcer production is between 0.10 N and 0.15 N.

*Distribution of the phosphatides in rat-liver nuclei and cytoplasmic particulates.* M. H. HACK (introduced by HUBERT R. CATCHPOLE). Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago.

Female white adult rats, maintained on an *ad libitum* diet of Purina Fox Checkers and water, were fasted 12-18 hours, killed by a blow on the head and the nuclei and cytoplasmic particulates isolated from pooled livers according to the procedures of Dounce (*J. Biol. Chem.* 147: 685, 1943) and Claude (*J. Exp. Med.* 84: 51-89, 1946) respectively. The phosphatide composition of the lyophilized nuclei, large-granules, microsomes and whole liver was determined by the method previously described (*J. Biol. Chem.* 169: 137, 1947). The results showed that as rats increased in weight (age) from 140 gm.-350 gm. the total phosphatide increased about 30% with the cephalin to lecithin ratio changing from 1:1-1:2. Assays run on

benzene extracts of the same tissues indicated that nearly half of the liver phosphatide was bound as a phosphatide-protein complex, mostly as lecithoprotein. Contrary to the observations of Stoneburg (*J. Biol. Chem.* 129: 189, 1939) and of Williams *et al.* (*J. Biol. Chem.* 160: 227, 1945) we have found nuclei to be the phosphatide-poor component of the cell. Our most homogeneous preparations showed the cephalin: lecithin ratio to be about 2:1, acetalphosphatide was absent and assays on benzene extracts indicated nearly 100% phosphatide-protein. The large-granule fraction seems to reflect most the liver phosphatide increase with growth which was due primarily to an increasing concentration of lecithin so that with increasing age the cephalin: lecithin ratio goes from 2:1 to 1:1. Acetalphosphatide was present and benzene extracts indicate that  $\frac{1}{3}$  to  $\frac{1}{2}$  of the phosphatide was bound to protein, largely as lecithoprotein. The phosphatide composition of the microsome fraction was found to remain quite constant with growth (140-350 gm. rats) with a cephalin: lecithin ratio of 1:1.4-1.6. About  $\frac{1}{3}$  was phosphatide-protein mostly of the lecithoprotein type. Acetalphosphatide was present in about the same concentration as in large-granules. However, there was present an unidentified 'acetal' which yielded a fuchsin addition product insoluble in capryl alcohol. Sphingomyelin was found to be either absent or of small concentration in whole liver and in the three cell fractions. The table shows the phosphatide distribution in the liver of 350 gm. rats. The data is expressed in  $\mu$ m/gm. dry weight of tissue.

	TOTAL PHOSPHATIDE	CEPHALIN	LECITHIN	SPHINGO- MYELIN
Whole liver.....	167.5	54.1	112.2	1.2
Nuclei.....	35.5	23.7	11.8	0
Large-granules....	229.2	107.3	112.7	9.2
Microsomes.....	321.0	129.2	191.8	0

*Effects of drugs and physical measures on spastic skeletal muscle.* NORMA M. HAJEK (by invitation) AND H. M. HINES. Dept. of Physiology, State University of Iowa, Iowa City.

A state of prolonged shortening or spasticity was produced in the gastrocnemius muscles of albino rats by the local injection of tetanus toxin. This condition was accompanied by atrophy and loss of strength in the affected muscles. Although temporary relaxation was elicited by curare injection, its frequent administration did not lessen the extent of atrophy and strength loss which occurred in spastic muscles. Treatment of shortened muscles by stretching reduced the amount of atrophy and strength loss. Curare facilitation did not enhance the beneficial effects of stretching. The results suggest that stretching spastic muscle without

curare is more beneficial. Excessive or 'over' stretching was found to be injurious to both normal and spastic muscles. Electrical stimulation of the spastic muscle in the fully stretched position resulted in less atrophy but greater strength loss per unit mass of muscle. Stimulation in the unstretched position proved to be ineffective.

*Some recent observations upon physiologic action of anti-histamine drugs.* B. N. HALPERN. French National Center of Scientific Research, Hôpital-La Charité, Paris, France.

Quantitative studies on capillary permeability have been made by following the passage of intravenously injected dye into the peritoneal cavity and into the anterior chamber of the eye. It has been shown that the antihistamine substance dimethylamino-2 propyl-L phenothiazine (phenergan) prevents the changes in rate of passage of the dye into the peritoneal cavity after peritoneal injection of histamine and that of fluorescein into anterior chamber after intravenous histamine. It has also been shown that this pheneoskiazine derivative prevents acute pulmonary edema in rabbits following intravenous epinephrine or chloropicrine. It is concluded that this antihistamine substance is a potent agent in preventing increase in capillary permeability produced by histamine and certain toxics.

*Effects of dihydroergocornine on the peripheral circulation in man.* D. W. HAYES, K. G. WAKIM, B. T. HORTON AND G. A. PETERS. Mayo Clinic and Foundation, Rochester, Minn.

The effects of the intravenous administration of dihydroergocornine (DHO-180), an alkaloid of ergot, on skin temperatures, blood pressure, heart rate and peripheral blood flow were studied in 20 human volunteers. The action of the drug is chiefly sympatholytic and, therefore, the drug is considered to be a vasodilator. It was administered to 6 patients by intravenous infusion in a solution containing 0.5 mg. of dihydroergocornine per 100 cc. of physiologic saline solution and to 14 patients by a single intravenous injection. The total dosage varied from 0.25-0.4 mg. Control values for skin temperatures, blood pressure, heart rate and blood flow were determined before the drug was given, and the observations were again recorded at regular intervals for a period averaging 65 minutes after administration of the drug. The blood flow was determined by means of a venous occlusion plethysmograph with a compensating spirometer recorder. Skin temperatures were recorded by means of thermocouples applied to the skin over the forehead, over the right and left deltoid muscles and over the right and left quadriceps femoris muscles.

Dihydroergocornine produced an over-all average increase in peripheral blood flow of 95% in the upper extremities, and 68% in the lower extremities, in 19 of 20 cases. In spite of the increase in blood flow in the extremities the skin temperatures were slightly decreased, even during the maximal increase in blood flow. The blood pressure fell in the 2 hypertensive cases after administration of dihydroergocornine. The decrease of systolic pressure was 58 mm. Hg in one case and 30 mm. Hg in the other, while the diastolic pressure fell 18 mm. in the former and 10 mm. of mercury in the latter. In normotensive subjects there was no significant change in blood pressure. The heart rate decreased in every case, with an average reduction of 13 beats per minute. Side reactions were more frequent than had been reported by other investigators, even with lower dosage. Nasal congestion, nausea, headache, flushing, an urgency for urination and vomiting were the side reactions observed.

*Rectal temperature patterns of dogs during peripheral vasodilation and vasoconstriction induced by the immersion method.* ALLAN HEMINGWAY. Dept. of Physiology, Univ. of Minnesota, Minneapolis.

Rectal and brain temperature measurements of normal unanesthetized trained dogs were measured during a testing process which consisted of local application of heat and cold sufficient to induce thermal cutaneous vasomotor activity. The animals were suspended in a hammock in a room at 15-17°C. and after a 30- to 45-minute rest the hind legs were immersed in water at 44 to 45°C. which was followed by immersion of the same limbs in cold water. Thermal cutaneous vasoconstriction and vasodilatation were indicated by changes in the skin temperature of the ear. A variety of rectal temperature patterns produced during this test was obtained. In some instances immersion of the hind limbs caused a sharp fall in rectal temperature when cutaneous vasodilation occurred, an effect which cannot be explained by the theory of the central control of body temperature. In other instances a rising rectal temperature occurred with vasodilatation, this being in accordance with the classical 'thermostat' central control theory.

*Cytology of the pituitary gland in experimental goiter.* GEORGE C. HENEGAR AND GEORGE M. HIGGINS. Mayo Foundation, Rochester, Minn.

With the discovery of the goitrogenic effects of certain thiourea and sulfone derivatives, study has been given to the influence these drugs exert upon the physiology of the thyroid. Although changes in the cytology of the anterior lobe of the pituitary exerted by thyroidectomy are well known, cytologic changes in the pituitary of animals made goitrous by these drugs



are less clearly understood. Studies were reported of the percentage distribution of cells comprising the anterior lobe of young male rats which had received certain well known goitrogens, promizole, thiouracil and thiobarbital. Correlations between the cytology of the pituitary, the degree of hyperplasia of the thyroid, and the basal metabolic rates were presented. The percentage of basophils in the anterior lobe of goitrous rats is greatly increased; the percentage of acidophils is decreased and large hyaline bodies, resembling those seen in the so-called 'thyroidectomy' cells are observed. Responses of thyroid and of anterior lobe to these three goitrogens were identical in kind but variable in degree. Thyroids were greatly increased in size; acinar cell heights were greater, and there was a marked decrease in colloid. Oxygen consumption was reduced. The pituitary-thyroid relationships were discussed.

*Cerebellar projections to the cerebral cortex in cat and monkey.* ELWOOD HENNEMAN, PAULINE COOKE AND RAY S. SNIDER (introduced by H. W. MAGOUN). Dept. of Anatomy, Northwestern Univ. School of Medicine, and Illinois Neuropsychiatric Institute, Dept. of Psychiatry, Chicago.

In 20 cats and 6 monkeys (chloralosan or barbiturate anaesthesia) single shocks were delivered to the cerebellum with electrodes applied to its cortex or placed stereotaxically in its nuclei. Amplified potentials, led from the cerebral cortex with bipolar electrodes, were photographed from a cathode ray oscilloscope. Depth of anaesthesia and physiological status were crucial. Excitation of anterior lobe in cat and monkey evoked surface positive monophasic potentials (10 sigma latency) in contralateral cerebral motor and sensory areas. Paramedian lobules projected bilaterally to sensory-motor areas I and II in cat. Both cerebellar auditory areas in cat projected bilaterally to cerebral auditory regions, responses centering around tip of anterior ectosylvian fissure (Garol's motor pinna area) and adjoining suprasylvian gyrus (Woolsey's motor-ear area). Elsewhere in the auditory cortex responses were inconstant. In some experiments on both species excitation of cerebellar visual cortex elicited cerebral visual responses (20-25 sigma latency). Nuclear stimulations evoked cerebral responses not confined within single functional areas. All sensory-motor and auditory areas responded in both species. In monkeys areas 6 and 8 responded discretely. In cats, but not in monkeys, the visual areas responded frequently. Sensory-motor, visual, and cingulate projections were distinguishable on the medial aspect of the cats' cerebrum. Thus, all investigated cortical areas having motor function receive cerebellar projections.

*Factors affecting the composition of alveolar air.* F. A. HITCHCOCK AND R. W. STACY (by invitation). Laboratory of Aviation Physiology, Ohio State Univ., Columbus.

The mass spectrograph developed in this laboratory for continuously recording the partial pressures of  $O_2$  and  $CO_2$  during the respiratory cycle makes possible the study of the dynamics of respiration. Curves obtained in normal respiration and during maximal exhalations have been analyzed. These curves show that a slight drop in the  $pO_2$  occurs before any change takes place in the  $pCO_2$ . A fraction of a second after this initial change there is a rapid drop in the  $pO_2$  and a still more rapid rise in the  $pCO_2$ , which accounts for almost the entire difference between inspired air and alveolar air. Following this there is a slow increase in the  $pCO_2$  and a slow decrease of  $pO_2$ , which continues until the flow of air out of the respiratory tract ceases. It appears that an important factor determining the partial pressure of alveolar air obtained by the Haldane-Priestly technique is the duration of the expulsion. During exhalation the  $pCO_2$  increases while the  $pO_2$  decreases at rates which may be as great as 2 or 3 mm. per second. Respiratory quotients calculated at  $\frac{1}{2}$ -second intervals during maximal exhalation show that the quotient during the first second is high, invariably being above 1. The value of the quotient is rapidly reduced and continues to drop as long as exhalation continues.

*Adequate cortical stimuli in the production of autonomic responses.* E. C. HOFF, A. C. JOHNSON, D. M. SHOLES AND E. H. GRAY. Medical College of Virginia, Richmond.

An experimental investigation of the characteristics of chemical and electrical stimuli which may evoke autonomic responses from the cerebral cortex has been carried out in the cat. Acetyl  $\beta$ -methylocholine chloride (Mecholyl, Merck) in saline solution, applied topically to the cortex produces profound falls in blood pressure with latencies within 15 sec. The threshold concentration for this response is lower, with shorter latency and greater magnitude, in the electrical pressor area of the cortex than in the electrical depressor area. Prior application of di-isopropyl fluorophosphate (DFP) to a cortical focus greatly lowers the threshold for the Mecholyl effect. In a typical experiment, DFP reduced the threshold concentration of Mecholyl from 2.5 mg/cc. to 0.3125 mg/cc. Topical application to the cortex of other cholinesters, including acetylcholine chloride, carbamylcholine chloride (Carcholin, Merck) and urethane of  $\beta$ -methylcholine chloride (Urecholin, Merck) likewise evoked sharp falls in blood pressure; but with these compounds no threshold-lowering effect of DFP could be demonstrated. Using



an electronic square-wave generator to deliver stimuli to the cortex, it was found that the threshold potential for autonomic responses is quite constant at 3 to 4 volts, the optimum range being reached just above threshold potential. Below threshold potential, no alterations of frequency or pulse duration produced a response. Frequencies of 30 to 180 cycles per sec. are within the optimum range and the extreme range is between 5 and 500 cycles. Pulse durations of 4 to 8 millisecon. apparently represent an optimum at a frequency of 60 cycles.

*Regeneration of nerve fibers to sweat glands.* W. HENRY HOLLINSHEAD. Section on Anatomy, Mayo Foundation and Clinic, Rochester, Minn.

Anomalous patches of sweating appearing in areas denervated by sympathetic chain ganglionectomy may be due either to failure to interrupt all the fibers supplying these areas or to subsequent regeneration. Since preganglionic fibers regenerate readily, and are cholinergic as are the postganglionic fibers to sweat glands, it is conceivable that sweat glands can be reinnervated by regeneration of preganglionic fibers. In order to test this possibility, the original cephalic end of the cervical sympathetic chain, after removal of the superior cervical ganglion, was anastomosed in the arm to either the median or the ulnar nerve. The flexor nerve not used for anastomosis was resected from the arm, but the radial nerve was left intact. The high skin resistances typical of the operated side began to decrease after the fourth month, and sweating thereafter reappeared on the previously denervated and dry foot pads. Section of the anastomosed nerve after sweating was well established failed to alter either sweating or skin resistance; subsequent section of the radial nerve again denervated the sweat glands. Thus functional regeneration was not due to regeneration of the anastomosed preganglionic fibers, but rather to growth from the intact radial nerve. Histologic examination of the anastomosed nerves indicated that the fibers of the sympathetic chain had entered the degenerated nerve in great numbers, but had failed to grow far enough along this to reach the level of the wrist, even though allowed ample time to do so.

*Effects of stimulation of the motor cortex after deafferentation.* J. HYDE (by invitation) AND E. GELLHORN. Laboratory of Neurophysiology, Univ. of Minnesota, Minneapolis.

Gellhorn (1948) observed the increased electromyographic EMG response to stimulation of the motor cortex which can be induced by fixating a joint to favor a muscle proprioceptively. The magnitude of this proprioceptive facilitation prompted an investigation to study the relative importance in the EMG response

of motor cortex and spinal cord. Muscle responses to cortical stimulation were studied before and after section of lumbo-sacral posterior roots. In 'Dial' cats the motor cortex was stimulated with condenser discharges. EMGs were recorded with an Offiner cryograph through copper wires sewn into semi-tendinosus and tibialis anticus. The latter muscle was tenotomized and its isometric tension recorded at different initial lengths. The limb was fixed, thus preventing lengthening of any intact muscles. Results obtained thus far indicate that slight variations in the frequency or intensity of motor cortex stimulation are capable of causing appreciable differences in the amplitude of EMG of muscles at a given initial length even after deafferentation has removed the possibility of proprioceptive recruitment. Further, confirmation of the idea that the EMG is dependent on the number of active motor units, whereas the tension developed is not (see Loof-borrow, 1948) was obtained under new conditions: in the control experiments, an increase in the initial length of tibialis resulted in an increased EMG as well as an increased tension in response to a given cortical stimulus; after deafferentation, however, while the tension increased with an increased initial length (as would be expected from the tension-length diagram), the EMG was unchanged by alterations in initial length of the muscle.

*Fluid shifts in animals during pressure breathing.*

CHESTER HYMAN AND JOSEPH GOODMAN. Dept. of Physiology, Univ. of Southern California, Los Angeles.

A satisfactory system for subjecting anesthetized animals to automatically controlled high pressure artificial-pressurized respiration has been developed. The system consists of a controlled source of pressure for inspiration and a similarly controlled loading against the expiratory pressure. The rate of respiration is determined by a telechron-operated switch which alternately opens inspiratory or expiratory solenoid valves. The magnitude of the pressures employed necessitates the use of proper protective counter-pressurization for the animals in order to prevent severe damage to the lungs. This protection is afforded by a system consisting of an air-tight bladder fitted to the animal and held in place by a non-elastic cloth jacket. A suitable pressure-tight helmet has been constructed to permit pressure breathing at these high levels without requiring direct tracheal cannulation. These garments and helmet are built to "pressurize" the entire thoracic and abdominal surfaces of the animal, but in no way protect the four limbs. Fluid loss from the circulation of animals exposed to pressure breathing under these conditions was determined by the degree of concentra-

tion of several blood constituents. One cc. samples were obtained from the exposed femoral arteries for the determination of hematocrit, hemoglobin, and plasma protein values. In addition, the concentration of previously injected non-diffusible dyes was determined. The general findings indicate a marked hemoconcentration, paralleled by a rise in the hemoglobin and plasma protein values. The fluid loss so determined was variable, but at least partially dependent on the level of pressure breathing maintained. The condition of the animal at the time of the experiment seems to play a major role in determining the final fluid loss. The fluid loss may be considered as a filtration of fluid from the abnormally high pressure in the capillaries into the more nearly normal pressures existing in the unprotected tissues of the limbs.

*Some observations on electromyography.* V. T. INMAN (by invitation), B. FEINSTEIN (by invitation) AND H. J. RALSTON. Univ. of California Medical School and College of Physicians and Surgeons, San Francisco.

Electromyographic studies of human voluntary muscle in cineplastic and normal subjects reveal the absence of measurable electrical activity in most muscles during rest, and even in various muscles of the lower extremity during certain phases of locomotion. The prevailing conception of muscle tone is questioned. Due to the existence of the length-tension diagram, there is no regular relationship between EMG amplitude and isometric tension, except at a given length of muscle. The use of electromyography in the study of phasic action of muscle groups is briefly discussed. The abrupt cessation of electrical activity in antagonists upon contraction of agonists is noted. It is shown that the amplitude of the EMG diminishes in the intact muscle as muscle length increases. This finding is in disagreement with the studies of Loofbourrow (*J. Neurophysiol.* 11: 153, 1948) on cortical stimulation of cats. Experiments on frog muscle-nerve preparations also do not agree with the findings of Loofbourrow on cat muscle, EMG amplitude increasing when the muscle stretched. The work of Duyff and Wiggers (*Arch. Neerl. Physiol.* 27: 195, 1943), showing decrease in chronaxie of isolated curarized, but not of isolated normal frog muscle under stretch, and decrease in chronaxie of intact muscle under stretch, is discussed in these connections.

*Theory of essential hypertension in man.* EDMUND JACOBSON. Laboratory for Clinical Physiology, Chicago, Ill.

As previously reported (A.A.A.S.), early essential hypertensive disease in man apparently can often be arrested by persistent application of progressive relaxa-

tion methods. Advanced cases commonly show persistently lowered pressure with lessened symptoms. Man differs from the experimental animal in that hypertensive disease arises in him during the efforts of struggle for existence. Efforts differ in races and cultures with the pace of living; if essential hypertension is a cultural disorder, medications, hormones, antirennin and other products may abate the symptoms yet fail to solve the basic problem. Muscle is the tissue by which man moves and imparts force to his environment. All effort, including so-called mental effort, proceeds by shortening of muscular fibers. Neuromuscular relaxation is freedom from effort, physical and mental. In relaxed muscle, most capillaries are closed. Upon contraction, they open in increasing numbers, due to action of metabolites. Increased blood pressure increases supplies and removes contraction products. Muscular contraction raises blood pressure through complex nervous reflexes and action of hormones and probably of metabolites. This effect often is masked. The glandular system possibly participates through nervous stimulation or through hormone action. The rôle of the kidney is still undetermined. Upon marked skeletal muscular contraction, renal arteriolar contraction (like retinal) increases, often to spasm. Renal anemia may increase production of angiotomin. Perhaps blood is shunted from the cortex (Truetta). Thus in early hypertension, arteriolar sclerosis is often absent (Smitchwick). Arteriosclerosis perhaps results from wear and tear especially from excessive effort. Individuals differ in hereditary susceptibility (Ayman). On this theory, the failure to reduce the death rate from hypertensive disease results from the failure to treat man as a self-directing individual.

*Ketogenic action of niacin and the alcohol of niacin.*

RALPH G. JANES AND I. M. PETERSON. Department of Anatomy, State Univ. of Iowa, Iowa City.

Earlier it was reported (*Soc. Exp. Biol. & Med.* 63: 410, 1946) that a ketonuria was produced in severely diabetic rats after relatively large amounts of niacin were added to their diet (1 g/kg. diet). It has been found in the present study that the alcohol of niacin when given in similar amounts was also ketogenic, but niacinamide was ineffective in producing a ketonuria. In rats with a milder diabetes, it was necessary, in certain cases, to feed high fat diets along with the niacin compounds in order to produce a ketonuria. When the high fat diet was continued but the excess niacin was withheld the urinary excretion of ketone bodies was reduced or disappeared, and reappeared only on the addition of excess niacin. However, two human diabetics were given niacin starting with 150 mg. daily and the dosage was slowly increased up to 1800 mg. without producing any untoward symptoms other

than the usual flushing reaction. The excretion of ketone bodies was not altered in these patients from pretreatment levels.

*Relation of blood sugar to spontaneous and insulin induced hunger sensations.* HENRY JANOWITZ (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Our recent studies have led us to believe that the gastric 'hunger' contraction and its epigastric pang are a dispensable component of the complex of hunger sensations in man. Because the rôle of blood sugar has been studied in the past only in relation to gastric contractions, the relationship of blood sugar to hunger sensations has been studied in five normal adult females in the post-absorptive state. 1) Spontaneous fluctuations of hunger sensations bear no relationship to minor variations in blood sugar level. 2) The intravenous injection of 0.3 gm. of glucose/kg. of body weight and the resultant hyperglycemia had no effect on spontaneous hunger sensations. 3) The intravenous injection of 0.1 U of insulin/kg. of body weight reproduced the pattern of spontaneous hunger, on the average, 38 minutes later, and 22 minutes after the depth of the hypoglycemia. The insulin induced hunger occurred at a blood sugar level of 45 mg. %, lasted 18 minutes, and subsided at a blood sugar level of 54 mg. %. It is concluded that blood glucose bears no relation to spontaneous hunger sensations, but that hypoglycemia may be operative under abnormal circumstances.

*Sensitivity to intocostarin of normal subjects, patients without myasthenia gravis and patients with myasthenia gravis.* P. S. JARRETT (by invitation), L. M. EATON (by invitation) AND E. H. LAMBERT. Sections on Neurology and Physiology, Mayo Foundation and Clinic, Rochester, Minn.

The effects of single intravenous injections of intocostarin or d-tubocurarine were studied on 17 normal subjects, 14 patients who did not have myasthenia gravis and 15 ambulatory patients who had myasthenia gravis. In terms of the equivalent dose of d-tubocurarine chloride pentahydrate/kg. body weight, the threshold amounts of drug producing a decrease in performance of the muscles tested varied from normal subject to normal subject in the following ranges: ocular convergence, 8.25 to 33.0; width of palpebral fissure, 33.0 to 99.0; strength of bite, 33.0 to 99.0; strength of grip, 33.0 to 132, and electromyographic response of abductor digiti quinti muscle (Harvey and Masland), 66.0 to 132. The sensitivities of normal subjects remained relatively constant in repeated tests. The gradient of sensitivities of different muscle groups was relatively constant from individual to individual in the

order in which the tests are named. The effects on nonmyasthenic patients were similar to those of normal subjects. All patients who had myasthenia gravis had a greater than normal sensitivity to curare in some muscle group or groups. In some instances, a decrease of performance was noted with doses as low as 1.4 micrograms per kilogram of body weight. Some patients who had myasthenia gravis had muscle groups in which the sensitivity to curare was not greater than that seen in some normal subjects. On 7 patients who had myasthenia gravis the relative sensitivity of different muscle groups did not follow the gradient of sensitivity found in normal subjects. This does not support the concept that a circulating curare-like substance is the sole cause of weakness in myasthenia gravis.

*Central neural mechanisms in cardiovascular depressor responses and sympathetic ocular changes resulting from stimulation of the cerebral cortex.* A. C. JOHNSON, E. C. HOFF, E. H. GRAY AND D. M. SHOLES. Medical College of Virginia, Richmond.

Observations of autonomic activity resulting from cortical stimulation were made as far back as the days of Hughlings Jackson. In recent years, largely through the impetus of Fulton and others, it has become obvious that autonomic nervous function is closely coordinated with other nervous activity, and is similarly arranged in a hierarchy of functional complexity culminating at the cortical level. We have shown that cardiovascular and ocular responses from the feline cerebral cortex are discrete, localized, and show definite patterns of functional localization. The area from which sympathetic responses are evoked includes the electrically-excitabile motor area and most of the frontal lobe cortex. From the cortex posterior to this pressor area as far back as the Sylvian fissure, falls in blood pressure have been elicited. Frequent rises in pressure after these falls suggest a close relationship to the pressor area. Ocular parasympathetic responses could never be produced by cortical stimulation. Vagotomy does not abolish these falls of blood pressure. Transcortical incisions separating pressor and depressor areas or frontal lobectomy likewise fail to abolish these depressor responses, although they are of smaller magnitude. Abolition of the secondary rises by such transcortical incisions implicates association pathways at the cortical level. Post-stimulatory ocular sympathetic responses, frequently evoked from the posterior portions of pressor area, suggest inhibition during the stimulus and subsequent release. The possibility that further developments may permit neurosurgical intervention at the cortical level in hypertensive cases is suggested.

*Influence of metabolic inhibitors upon membrane potentials in vivo.* ERVIN KAPLAN AND NORMAN R. JOSEPH (introduced by C. I. REED). Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

The membrane potential across the synovial membrane of the dog was determined *in vivo*. Under the influence of metabolic inhibitors, a positive potential was produced at the site of inhibition. Potentials were measured from the interior at the knee joint to the subcutaneous tissue overlying the medial condyle of the femur. (See Joseph, N. R. *et al.*, *Am. J. Physiol.*, 153: 364, 1948.) Standard readings were obtained with 0.15 M. NaCl on both sides of the capsule prior to the intra-articular application of an inhibitor solution. Neutral, isotonic solutions of the inhibitors in NaCl were employed in all instances. Substances which produced positive potentials of varying magnitude included the following: Na<sub>2</sub>S, NaCN, Na azide, Na iodacetate, Na malonate, hydroxylamine, p-chloro-mercurobenzoate, HgCl<sub>2</sub>-ferric and cupric ions. The cytochrome-cytochrome oxidase system is the principal enzyme complex involved in electron transfer without hydrogen transfer. Therefore, variations in observed potentials may be interpreted in terms of electron transference and potential differences in the cytochrome system. Variations in potential and electron flow may be produced by changes at any point in the transfer systems. These alterations may take place at the substrate, the dehydrogenase systems, the hydrogen-electron transference systems or the cytochrome system. In specific inhibitions the level of the cytochrome oxidase potential may be decreased; in other instances, the cytochrome system may be partially or completely bypassed with hydrogen-electron transfer via alternate pathways.

*Effect of massage and preliminary warming-up upon athletic performance.* PETER V. KARPOVICH AND CREIGHTON J. HALE (by invitation). Physiology Dept., Springfield College, Springfield, Mass.

It is almost axiomatic with coaches and athletes that a preliminary warming-up is indispensable with a good performance. Two reasons are usually given: the strength and speed of muscular contractions increase and the muscles and tendons 'limber up' and increase resistance for possible injuries which may result from sudden intensive muscular effort. On the other hand, one may meet athletes who either use no special warming-up or gave up its use because they could not see any advantage in this procedure. Laboratory experiments conducted by Asmussen and Bøje showed that preliminary work, diathermy and hot showers increased capacity for riding a bicycle ergometer; massage on the other hand had no effect. Schmidt found that in riding bicycle ergometers, running 100 meters, or swimming 50 meters, all methods used by Asmussen and Bøje

were beneficial. The present investigators were especially interested in the controversy regarding massage. For this purpose, they experimented on 7 college runners using three types of warming-up: 1) preliminary exercise (10 min.); 2) massage (10 min.); 3) light digital stroking (5 min.). The last method was used as a control, involving a possible psychological effect. Immediately after either of these procedures the subjects ran 440 yards. Each type of warming-up was repeated on each man from two to three times. The average time of 20 trials after preliminary exercise was 56.17 seconds; after massage, 55.84 seconds; after digital stroking, 56.50 seconds. Statistical computations showed that the differences between these times were not statistically significant. Additional experiments are in progress.

*Changes in the blood following explosive decompression.*

J. P. KEMPH (by invitation) AND F. A. HITCHCOCK. Laboratory of Aviation Physiology, Ohio State Univ., Columbus.

Determinations of blood gases, hematocrit, blood cell morphology, mean corpuscular hemoglobin concentration and intravascular bubble formation have been made following explosive decompression of dogs. Results show an increased hematocrit which may have been caused by one or more of the emergency measures which increase blood concentration. No significant change was noted in the formed elements of the blood, nor in mean corpuscular hemoglobin concentration. There is therefore no evidence of a water shift between the plasma and the red blood cells or of intracellular bubble formation. No intravascular bubbles were noted 30 seconds after explosive decompression to barometric pressures greater than 55 mm. Hg; bubbles were a frequent finding within 30 seconds at lower barometric pressures and a constant finding after one minute exposure to 30 mm. Hg. Bubbles were noted in superficial arteries within 30 seconds and in veins within 3 minutes after explosive decompression to 30 mm. Hg. Therefore it was thought that intravascular bubbles were formed centrally rather than peripherally; i.e., in the large veins, heart or pulmonary system. The circulation was probably blocked by these bubbles. Determinations of blood gases showed considerable oxygen present after explosive decompression to pressures less than 50 mm. Hg. Therefore, stagnation anoxia and other factors, rather than anoxic anoxia, may be significant in the etiology of the effects of rapid decompression to barometric pressures below the vapor pressure of body fluids.

*Quantitative measurement of regional circulation by the clearance of radioactive sodium.* SEYMOUR S. KETY (introduced by JULIUS H. COMROE, JR.). Univ. of

Pennsylvania, and Diabetic Acidosis Project, Phila. General Hospital, Philadelphia.

A freely diffusible substance injected into a living tissue will be carried away from the site of injection at a rate largely determined by the local circulation. If the substance be radioactive, the quantity remaining in the tissue, and hence the clearance rate, may be measured continuously by means of an appropriately shielded Geiger-Müller counter with a fixed geometrical relationship to the injection site. It was theoretically predicted and experimentally found that the quantity of sodium remaining in the tissue ( $Q$ ) at time ( $t$ ) after injection would bear a simple exponential relationship to the initial quantity injected ( $Q_0$ ):  $Q = Q_0 e^{-kt}$  where  $k$  represents a tissue clearance constant. It is further demonstrable that  $k$  bears the following relationship to the tissue blood flow:  $k = \frac{F}{S} \theta$ , where  $F$  =

tissue blood flow as cc/gm/min.,  $S$  = sodium space of the tissue as cc/gm. and  $\theta$  (numerically somewhat less than unity) = an overall diffusion factor depending on diffusion distances, capillary interface, fluid filtration and absorption. To test the responses of the method to procedures expected to alter the local circulation, measurements were made in the human gastrocnemius muscle at rest, during arterial occlusion and reactive hyperemia, before and after exercise of the foot, and before and after the local injection of a vasoconstrictor drug. In each case the clearance constant followed the expected changes in circulation. The method appears to offer a convenient and clinically useful means for the quantitative measurement of effective circulation in a particular tissue.

*Water and air densitometry.* A. KEYS AND J. BROZEK. Univ. of Minnesota, Minneapolis.

Gross density of the human body is the resultant of the proportions of the contained extracellular water, bone mineral, fats and the remainder which may be termed the active cell mass (A.C.M.). Bone mineral (sp.gr. 2.0) accounts for about 4% of the total body mass. Extracellular water (sp.gr. 1.01) may be separately estimated (e.g. with CNS) and is roughly proportional to the A.C.M. except in disturbed water balance. The sp. gr. of the A.C.M. is roughly 1.02. Measurement of the gross sp. gr. allows estimation of the proportions of the several components. Densitometry by weighing the body in air and under water is demonstrated. The result must be corrected for residual air in the lungs. Body volume may also be estimated by its effect on the pressure relations in a closed air system of known size. Apparatus for this is demonstrated. With quiet breathing the body volume measured in air is smaller than the water displacement

by an amount equal to the residual air. Theoretically, the air pressure system also allows estimation of gas in the digestive tract by measuring total body compression at different known pressures.

*Use of an improved photo-electric plethysmograph for determination of blood loss from the ear on the human centrifuge.* JULIAN R. B. KNUTSON (by invitation) AND EARL H. WOOD. Section on Physiology, Mayo Foundation, Rochester, Minn.

The multiple photo-electric cell in the modified oximeter earpiece described by Wood and Geraci has been replaced with a single photocell covered with a double thickness of Wratten No. 88A infrared filter. The spectral response characteristics of iron-selenium barrier layer photo-electric cells coupled with the transmission characteristics of the 88A filter render this combination selectively responsive to light of wave lengths in the region of 800 millimicrons. The absorption of light of this wave length by whole blood is dependent on the hemoglobin content of this blood, and is independent of the degree of oxygen saturation. A translucent pneumatic pressure capsule interposed between the light source and the ear makes possible measurement of the transmission of infrared light of the bloodless (pressurized) ear and of the normal blood-containing ear. The difference in transmission of light between the bloodless and blood-containing ear is a measure of the infrared transmission of the blood alone interposed in the optical path of the earpiece, and hence of the amount of blood (hemoglobin) contained in this portion of the ear. This photo-electric plethysmograph has been used to determine the percentage of blood lost from the ear during 134 fifteen-second exposures of ten subjects to positive accelerations ranging from 2.1 to 5.1 g. The percentage of the control (1 g) blood content of the ear which was lost during exposures to accelerations that produced complete loss of vision was  $57 \pm 2.3$ ; loss of peripheral vision, only  $48 \pm 2.9$ ; dimming of vision,  $39 \pm 2.8$ ; and no impairment of vision,  $30 \pm 2.1$ . In these subjects inflation of the capsule in the earpiece at 1 gm. to pressures of 20, 50, 100, 150 and 200 mm. Hg produced average decreases in blood content of 47, 84, 98, 100 and 100%, respectively. Therefore, the intracapsular pressure required to produce the maximal transmission of infrared light by that portion of the ear interposed in the optical path of the earpiece is approximately equal to the systolic arterial pressure at the ear. The percentage of blood lost from the ear per unit change in acceleration was calculated for each exposure. This value tended to be independent of the g level of the individual exposures, and to vary significantly from subject to subject. The average values obtained for individual subjects averaged 18, and ranged from 10

$\pm 0.6$  to  $25 \pm 0.7\%$  per  $g$  change in acceleration. There was a positive correlation from subject to subject between the per cent of blood lost from the ear and the degree of visual symptoms produced by exposures to acceleration. This correlation was somewhat better than that found between visual symptoms and the per cent reduction in ear opacity pulse, and was inferior to the correlation between systolic blood pressure at head level and visual symptoms, obtained by Lambert and Wood in another series of subjects.

*Effect of microwave diathermy on the peripheral circulation and on tissue temperature in man.* A. J. KOSMAN, L. L. SIEMS AND S. L. OSBORNE. Northwestern Univ. School of Medicine, Chicago, Ill.

It has been universally accepted that local heating of an extremity increases the blood flow to it. Recently, Kemp, Paul, and Hines made the interesting observation that although blood flow in the femoral artery of the dog is increased by microwave diathermy, heating with short wave diathermy produced little or no change in blood flow. Confirmation of these results would necessitate a complete revision of the present concepts of the physiological effects of heating. In the present investigation, the effect of both types of heating upon femoral blood flow were studied in a series of normal dogs and dogs with an unilateral denervation of the hind extremity. The animals were maintained under pentobarbital anesthesia in a room kept at  $30^{\circ} \pm 1^{\circ}\text{C}$ . The femoral arteries were cannulated bilaterally and the cannulae attached on each side to "bubble flow meters" which were slight modifications of the design of Leden, Herrick, Wakim, and Krusen. Flow readings were made bilaterally and simultaneously. To prevent clotting the animals were given 100 mg. of oral dicumarol 24 hours before the experiment and 100 mg. of heparin intravenously at the time of the experiment. The temperature of the gastrocnemius muscle was measured before and after heating by means of a copper constantan thermocouple. These readings were taken only to be certain that a fair degree of heating had taken place, since, of course, temperature change depends not only upon the energy absorbed but also upon the rate of heat dissipation by the changing blood flow. Microwaves (frequency of 24.50 m.c.) were applied by means of a 'four-inch director' placed 2 inches from the skin. Short wave (induction method) diathermy (frequency of 27.33 m.c.) was applied by means of a standard hinge-jawed drum adjusted to surround the leg. In both cases after a one-hour control period, heating was directed to the region of the gastrocnemius for 20 minutes. The dogs with unilateral denervation of the hind extremity had one of the 3 following lesions: 1) section of ventral roots  $L_4$  to  $L_7$ , inclusive; 2) section of dorsal and ventral roots  $L_4$  to  $L_7$ , inclusive; 3) section

of the sciatic, femoral and obturator nerves peripherally. The blood flow studies were carried out 12 weeks after the production of the lesion. The experimental animals represented three distinct types of lesions: 1) pure motor loss; 2) combined motor and sensory loss; and 3) motor, sensory and autonomic loss. To compensate for any distortion resulting from spontaneous fluctuations in blood flow, flow changes were analyzed on the basis of the difference between the control (unheated) and heated side just before and at the end of the heating period. Statistical significance was determined by the method of paired comparisons. An examination of the first slide reveals that there is a significant increase in blood flow upon the application of either form of heating in both normal and root lesion animals. These differences are significant at the 5% level of probability. Due to the limited number of root lesion animals the results are grouped for analysis and presentation according to the type of heating. In all cases the response is essentially the same. In dealing with the peripheral nerve lesion group, it became obvious that the response typical of the other animals could not be obtained. Since there were not enough dogs in the peripheral nerve lesion group to permit separate analysis of microwave and short wave heating, and since it was quite clear that both methods of heating were equally effective in the other groups, for purposes of comparison the animals were arranged according to the type of lesion represented. Although the response of the root lesion dogs to heating is essentially the same as in normal dogs, ( $t$  ratios significant at the 1% level of probability), significant increases in blood flow could not be obtained in those animals subjected to peripheral nerve section. To summarize, these experiments clearly demonstrate that short wave and microwave diathermy are equally effective in producing an increase in blood flow in the hind extremities of normal dogs. These results do not support the thesis that a particular method of heating may possess properties which exert a specific effect upon blood flow. Although loss of motor and/or sensory innervation to the extremity does not change the essential nature of the vascular response to heating, the behavior of the peripherally denervated animals suggests that the integrity of the sympathetic outflow in the dog is necessary for the typical vascular response to heating.

*Electrolytic changes in fibers of voluntary muscles of rats as revealed by the microincineration technic.* EDWARD M. KRUSEN AND H. E. ESSEX. Mayo Foundation, Rochester, Minn.

The microincineration technic developed by Scott was adapted to study changes of mineral ash in the fibers of muscles subjected to various experimental conditions. These conditions included: 1) exercise

(swimming with attached weights), 2) electrical stimulation, 3) anoxia produced by the application of tourniquets, 4) denervation and 5) tenotomy. The results indicated that the fibers of muscles exercised showed an increase in density of mineral ash occurring after definite periods of contraction. The degree of increase in density of mineral ash was found to be proportional to the strength and duration of the activity up to a certain limit. Once a certain degree of increase had occurred, activity for longer periods caused no further changes. The ash of muscle fibers serving as controls was gray in color; the ash of muscle fibers in which an increase occurred was chalky white. Results with electrical stimulation were similar to those obtained with exercise. The fibers of muscles stimulated electrically showed an increase in density of mineral ash occurring after definite periods of contraction, regardless of the method of stimulation. Anoxia produced by prolonged application of tourniquets resulted in little change in electrolytes. Denervation and tenotomy produced a slight initial increase, followed by a decrease in the density of mineral ash in the fibers of the affected muscles as compared with fibers of normal muscle. The color of the ash in the later stages was blue, contrasted with the gray ash of the fibers of normal muscles.

*Physiologic limb preference.* ELEANOR M. LARSEN (introduced by WALTER J. MEEK). Dept. of Physiology, Univ. of Wisconsin Medical School, Madison.

The extensive cortical areas controlling limb movement would indicate important physiologic significance. The hand motor area is greater than that for the foot. Unilaterality and bilaterality are exhibited from molecule to man. The incidence of human limb preference is unknown. The majority of individuals demonstrate right-handed preference for *certain* responses, but left-handedness has been observed and stated as occurring in from 10 to 25% of the population, suggesting Mendelian ratio. There are 3 reactions for hands and feet: right, left, or bilateral, forming 8 preference patterns. To determine physiologic limb preference an electronic modification of a precedence indicator was employed. The hands, or feet, were placed upon telegraph keys which controlled thyatron's operating electro-magnetic counters. Upon appropriate light stimulus the pair of limbs were lifted as simultaneously as possible. If one extremity reacted within 10 micro-seconds prior to the other, its precedence was recorded. One hundred effective stimuli were given in 4 complex bouts with rest periods intervening. The hands and feet of 62 healthy young adult women were tested. The physiologic hand reaction generally reflected the usual response, but individuals *changed* in childhood

often revealed the original preference, although they wrote with the educated hand. The results indicate that the majority of individuals are physiologically bilateral in reaction and only a small proportion are significantly right or left in preference. The data suggest that the nerve pathways for limb stimulus-response may be more efficient either bilaterally, or unilaterally, and this neurophysiologic basis determines intrinsic, or physiologic preference.

*Analgesia and anesthesia induced by epinephrine.* A. LEIMDORFER. Dept. of Psychiatry, Illinois Neuropsychiatric Inst., Univ. of Illinois, and Dept. of Pharmacology, Loyola Univ., School of Medicine, Chicago.

Observations during our earlier experiments about analgesia following intracisternal, (i.e.) injection of epinephrine (epn) and reports of Ivy and co-workers (about analgesia after intracarotid injection of epn) suggested the trial of epn intrathecally for surgical anesthesia. Twenty-six experiments were performed on dogs. Usually pure epn powder (Parke Davis) dissolved in water was used. In some experiments, epn alone was injected ( $\frac{1}{2}$  or 1-2 mg/kg) without sedatives. Several minutes after the injection, the dogs became quiet and, about  $\frac{1}{2}$  hour later, they were asleep (for 1-2 hr.). Cutting the skin and complete laparatomies were performed during which no signs of pain were observed. In other experiments, a small amount of nembutal was injected intraperitoneally (as a kind of basal anesthesia) prior to epn to decrease the amount of epn needed. Several hours after nembutal, the dogs awakened and showed distinct signs of pain already on pinching the skin. At this time, epn was injected ( $\frac{1}{2}$  mg/kg.). About 30 minutes later, complete surgical anesthesia occurred. The bloodpressure, ECG and EEG remained normal, the respiration was not depressed, but stimulated. No after effects were seen during the time of observation after the injection (for 2-3 months) although three to four injections were made into the same dogs. However, two dogs died after extremely large doses. No analgesia and no sleep were observed after i.c. ephedrine. Intradermal injection of epn (1 cc. of 1:20,000) (guinea pigs) produced local anesthesia.

*Absence of increased insulin sensitivity of eviscerated adrenalectomized rats.* R. LEVINE, B. SIMKIN AND D. CUNNINGHAM. Dept. of Metabolic and Endocrine Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

It is a well established fact that intact adrenalectomized animals are very sensitive to the hypoglycemic effect of insulin. The C<sub>11</sub>-oxy-steroids on the other hand oppose this action of insulin. In order to delimit



the locus of this hormonal interaction, we have compared the insulin sensitivity of adrenalectomized, eviscerated, liverless rats with the sensitivity of normal eviscerated control animals. The data show that the minimum dose of insulin necessary to effect significantly the blood sugar of either group lies between 0.15 and 0.2  $\mu$ /kg. body weight. That is, in the absence of the liver, adrenalectomy no longer causes an increased sensitivity to insulin. It would seem therefore that the insulin sensitivity of the *intact* adrenalectomized rat is due to the unopposed action of insulin on the viscera, most probably the liver, and not to unhindered peripheral (or muscle) action. These data emphasize again that the primary carbohydrate abnormality of the adrenalectomized animal is a deficient hepatic sugar production.

*Metabolism of acetate by the isolated dog gastrocnemius investigated with carboxyl-labeled acetate.* NATHAN LIFSON, AKIRA OMACHI (by invitation), H. MEAD CAVERT (by invitation) AND JOHN A. JOHNSON (by invitation). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

The isolated gastrocnemius of an etherized dog was perfused by means of a closed, pump-oxygenator circuit, the animal's own heparinized blood serving as the perfusion fluid. The muscle was stimulated via the sciatic nerve with maximal single shocks at a frequency of 1/sec. for 75-100 minutes. After the anesthetic ether had been removed (or nearly so) by aeration, carboxyl-labeled  $C^{13}$ -sodium acetate was administered to give a blood level of approximately 1.8 mM/100 cc. In 3 experiments it was calculated (from:

$$\frac{\text{atom } \% C^{13} \text{ excess in respiratory } CO_2}{\text{atom } \% C^{13} \text{ excess in administered acetate}} \times 100$$

that 26-49% of the respiratory carbon dioxide was derived from the administered acetate on the assumption that both carbon atoms of acetate are converted to  $CO_2$  at equal rates. If only the carboxyl carbon were so converted, these values would be reduced by one half. In 2 control experiments in which a rubber tube was substituted for the muscle, relatively trivial amounts of isotope appeared in the 'respiratory' carbon dioxide. Net disappearance of acetate as measured by blood volatile acid determinations was considerable, amounting to approximately 0.9-2.5 mM in the muscle experiments, on the assumption that the acetate of the blood-muscle system is uniformly distributed. In the controls no disappearance of blood acetate was detected. No definitely significant excess  $C^{13}$  was found either in the blood lactate at the end of the experiment or in the muscle glycogen. An accumulation of formate in the blood was not demonstrated. From the above data it is concluded 1) that contracting mammalian

skeletal muscle can convert at least the carboxyl carbon of acetate to carbon dioxide and 2) that, in all probability, such muscle can effect net disappearance of acetate.

*Effects of postganglionic sympathectomy and vagotomy upon the gastrointestinal tract.* C. WALTON LILLEHEI, Dept. of Surgery, Univ. Hospital, Univ. of Minnesota, Minneapolis.

Following complete removal of the celiac, superior, and inferior mesenteric ganglia in dogs, there invariably occurs a fulminating diarrhea associated with bloody stools and the passage of increased amounts of mucus. Extirpation of these ganglia, also referred to as prevertebral ganglionectomy, removes essentially the postganglionic sympathetic fibers from the stomach, small intestine and colon and results in a high mortality in the dogs within the first 2 to 3 weeks due to the resulting inanition and to the pathological changes which occur such as severe gastrointestinal hyperemia, hemorrhagic enteritis, colitis, and in  $\frac{1}{4}$  to  $\frac{1}{2}$  of the dogs the development of typical appearing peptic ulcers located for the most part in the stomach and duodenum, but occasionally as far down as the terminal ileum. These ulcers likewise often progressed to hemorrhage or perforation. The simultaneous excision of the vagi nerves along with prevertebral ganglionectomy prevented in all animals the development of peptic ulcers and gastroenteritis. The essential physiological changes following postganglionic sympathectomy of the gastrointestinal tract are discussed and compared with the effects of preganglionic sympathectomy upon the gastrointestinal tract.

*In vivo iodination of tissue protein following injection of elemental iodine.* H. J. LIPNER AND S. B. BARKER, Dept. of Physiology, State Univ. of Iowa, Iowa City.

Subcutaneous injection of elemental iodine, dissolved in propylene glycol, caused an elevated plasma protein-bound iodine (PI) in normal, thiouracil-treated or thyroidectomized rats. At least some of the PI appears to be thyroxine, since the metabolism of the hypothyroid animals was increased during the period of the injections. The possible rôle of various organs of the animal body in the elaboration of the PI was investigated by comparing the tissue PI levels for kidney, liver, muscle, heart and thyroid with that found at the site of injection. There can be little doubt that iodination of tissue protein took place primarily at the site of the iodine injection, since this PI value was several hundred times greater than any other. The concentration of PI in the kidney was elevated to about the same extent as in plasma, but the liver to a considerably smaller extent. In addition to not being necessary for the iodination reaction being studied here, the thyroid gland appears not to participate, even when present.



*Proprioceptive reflexes and muscle coordination.* G. N. LOOFBOURROW AND E. GELHORN. Laboratory of Neurophysiology, Univ. of Minnesota, Minneapolis.

The same functional associations of limb muscles found by Bosma and Gellhorn to respond to stimulation of specific sites in the monkey's motor cortex may be activated on a spinal level by muscle stretch. By means of electromyography it may be demonstrated that the triceps and the flexors carpi show a reflex contraction as a result of passively flexing the elbow. After tenotomizing the triceps, neither it nor the flexors carpi respond to elbow flexion, showing that the response of both depends upon proprioceptive reflexes originating in the stretched triceps. A pull on the tendon of the triceps again elicits activity in both muscles. Stretching the flexors carpi, either by dorsiflexion of the wrist or direct load, excites the same complex. The biceps-extensor complex is activated by elbow extension, even after tenotomizing the extensor carpi. Stretching an extensor carpi by a load on its tendon, or by volar flexion of the wrist also excites the complex. If a reflex be evoked by electrical stimulation of an afferent nerve, it too excites one complex or the other, depending on the nerve chosen. Facilitation of the response of the 'associated synergists' in such a complex results from proprioceptively 'favoring' one or more muscles in it. The 'favoring' has been done by loads applied individual muscles, and by fixation of joints in a position to place certain muscles under stretch. This facilitation has been shown to hold for the semitendinosus-tibialis complex in the hind leg, as well as for the biceps and triceps complexes.

*Conversion of lactate to liver glycogen in the intact rat, studied with C<sup>13</sup>-labeled lactate.* VICTOR LORBER, NATHAN LIFSON, HARLAND G. WOOD (by invitation) AND WARWICK SAKAMI (by invitation). Dept. of Biochemistry, Western Reserve Univ., Cleveland, Ohio, and Dept. of Physiology, Univ. of Minnesota, Minneapolis.

The metabolic path linking lactate and liver glycogen has been investigated in the intact rat. C<sup>13</sup>H<sub>5</sub>C<sup>13</sup>-HOHCOONa and CH<sub>3</sub>C<sup>13</sup>HOHCOONa were given with glucose by stomach tube to fasted rats. The resulting liver glycogen was isolated, hydrolyzed to glucose, and the position of the C<sup>13</sup> in the glucose molecule determined (*J. Biol. Chem.* 159: 475, 1945). The distribution of isotope in the glucose is indicated in the table. Values are in atoms % excess C<sup>13</sup>.

TYPE OF LACTATE FED	C <sup>13</sup> IN THE GLUCOSE FRACTIONS		
	carbons 3,4	carbons 2,5	carbons 1,6
CH <sub>3</sub> C <sup>13</sup> HOHCOONa....	0.05	0.27	0.18
C <sup>13</sup> H <sub>5</sub> C <sup>13</sup> HOHCOONa...	0.15	0.60	0.59

The distribution of isotope in the glucose in both

types of experiment is consistent with the reactions of glycolysis and the tricarboxylic acid cycle as the main paths for the conversion of lactate to glycogen. Interpreted on the basis of these reactions, the distribution of isotope in the 1,6 and 2,5 carbons of the liver glucose in the α-labeled lactate experiment

.18 .27 .18  
(C·C·C·C·C·C) results from the summing of two

.09 .09  
types of isotopic glucose, (a) C·C·C·C·C·C, and (b)

.18 .18 .18  
C·C·C·C·C·C, where the superscript actually represents the relative contribution of excess C<sup>13</sup> made to the final glucose. Glucose (a) is formed directly via the reversal of glycolysis. Glucose (b) is formed from lactate which, via pyruvate and CO<sub>2</sub> fixation, has entered the tricarboxylic acid cycle. Four times as much isotope is seen to have traversed the latter path as the former. Assuming that glycolysis and the tricarboxylic acid cycle are the main pathways of conversion, four mols of administered lactate traverse reactions of the tricarboxylic acid cycle prior to glycolysis for each mol that is converted to glucose directly via glycolysis.

*Effect of acute anemia, of anoxemia and of convulsions upon the temperature of the hypothalamus of the cat.* HANS LOWENBACH. Depts. of Neuropsychiatry and Physiology, Duke Univ. Hospital and School of Medicine, Durham, N. C.

Thermo-electric measurements were made in the brain of 43 cats under light nembutal anesthesia. Compression of the common carotid artery, either on one side or on both sides simultaneously, produced a prompt and marked increase of the temperature in the hypothalamus and adjacent rhinencephalic structures. The temperature rose usually about ½ of 1°C. within two minutes, but occasionally greater changes were observed. In the thalamus and in other cerebral tissue above the hypothalamus, the temperature increase was smaller and it was delayed in proportion to the distance from the hypothalamus. In and near the cerebral cortex, the temperature fell during occlusion of the carotid artery and rose after readmission of blood. Asphyxia and convulsions induced by various means, produced a comparable increase in heat production in the hypothalamus.

*Effect of antihistamine drugs on the flares produced in the skin of normal human beings by burning and freezing.* ALEXANDER LOWY AND CHARLES F. CODE. Mayo Clinic, Rochester, Minn.

Lewis and his co-workers have shown that a triple response involving the production of 1) a red spot, 2) a wheal at the site of the red spot and 3) a surrounding

flare is the standard stereotyped response of the skin to minor injury and that the response is due to the liberation of an H-substance which is either histamine or a substance closely resembling histamine. As a means of further elucidating the mechanism of this reaction to injury we have studied the effects of antihistamine drugs on the flare component of the triple response produced by burning or freezing the skin of normal human beings. The flares that we obtained in response to burning were small and the antihistamine drugs had no effect on them. Because of the minimal response that we obtained, definite conclusions regarding the action of these drugs on burning are not being drawn. The results obtained with freezing however were decisive. Large, uniform flares were routinely obtained. These were consistently inhibited by the presence of antihistamine drugs.

*Prophylaxis and therapeutics of experimental renal hypertension with purified renin.* J. M. MARSHALL (by invitation), HIROAKA MINATOYD (by invitation), R. O. BURNS (by invitation) AND G. E. WAKERLIN. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Previous studies of the effects of crude hog renal extracts on renal hypertension in the dog have shown that the injection of certain renin-containing extracts could prevent the development of renal hypertension following renal artery constriction and could reduce the blood pressure in dogs with an already established hypertension. Antirenin was produced by these extracts, but correlation of antirenin with antihypertensive effect has not been established. In the present study a more highly purified preparation of hog renin was used, both in prophylaxis and in therapy, to determine whether renin was the active agent in crude extracts and to investigate the rôle of renin in the initiation and maintenance of renal hypertension. Hog renin was prepared by a controlled ethanol fractionation technique. Purity of successive lots ranged from 35 to 125 DU (Goldblatt) per mg. nitrogen, averaging about 75. Blood pressures were determined twice weekly by direct femoral puncture. Antirenin titers of serum were determined at monthly intervals. Ten dogs were used in a prophylaxis experiment. After a control period of pressure determinations the dogs were injected daily intramuscularly with purified hog renin. Seven dogs received 10 DU/kg. daily, and 3 received 3 DU/kg. After 3 months the right renal artery was constricted by a Goldblatt clamp. Three weeks later the left renal artery was constricted. Renin injections were continued for 1 month following the second clamping, in surviving dogs. As a control group, 9 normal dogs were bilaterally clamped in exactly the same

fashion. Results in the 2 groups differed markedly. All 9 controls became hypertensive, giving elevations of 30 to 60 mm. Hg. in the first week following the second clamping. These dogs had only mild transient impairment of renal function (maximum blood urea nitrogen values below 60 mg. %). The 10 dogs treated prophylactically showed severe renal insufficiency after the second renal artery constriction, with values of 60 to 250 for b.u.n. Seven of the 10 died in 3 to 9 days in typical renal insufficiency. Three survived and recovered adequate renal function without elevation of blood pressure. Of the 10, only 2 showed any rise in pressure (20 and 40 mm.). These had low antirenin titers (6 DAU/ml.). The maximum antirenin titers of the other 8 dogs ranged from 3-120 DAU/ml.

*Production and closure of atrial septal defects in the dog: Observations on atrial pressures.* WILLIAM B. MARTIN AND HIRAM E. ESSEX, Div. of Experimental Medicine, Mayo Foundation, Rochester, Minn.

A procedure was devised in dogs which resulted in large atrial septal defects. A knife was introduced into the right atrium through the auricular appendage, the tip advanced to the septum and this structure incised. Right atrial pressures taken by a water manometer averaged 3.8 cm. in a series of 9 normal dogs with open chests. Left atrial pressure in a series of 7 normal dogs with open chests averaged 6.3 cm. H<sub>2</sub>O. In 6 animals in which the pressure in both chambers was measured, the left reading exceeded the right by an average of 2.6 cm. H<sub>2</sub>O. In a series of 5 animals with proved septal defects in which both right and left pressures were recorded within a few minutes of each other the left exceeded the right by an average of 4.3 cm. H<sub>2</sub>O. In 3 of 4 dogs with proved atrial septal defects the right mid-atrial sample of blood exceeded the anterior vena cava sample by 2.5, 5.1 and 5.9 volume % of oxygen. For closing the atrial septal defects a piece of polythene sheeting covered with an inverted segment of vein was introduced into the right atrium and brought into apposition to the atrial defect. Three silk sutures with straight needles were attached to the graft and introduced separately into the right atrium through a small incision. The needles were brought out of the chamber at points in the plane of the atrial septum. The graft became endothelialized within two weeks. The atrial defect closed spontaneously behind the graft within one month.

*Effect of prostigmine on intestinal motility in human beings.* JOHN M. MCMAHON, CHARLES F. CODE, WILLIAM G. SAUER AND J. ARNOLD BARGEN. Mayo Foundation and Clinic, Rochester, Minn.

A study has been made of the effect of prostigmine on the motility of the bowel in a series of human volunteers.

Detailed observations were carried out on 5 subjects. One of these had undergone ileostomy and 4 had undergone colostomy. The motility of the bowel was recorded by means of a tandem balloon system connected with a glass-spoon manometer which recorded optically on a photographic camera. The balloon system contained both water and air. The recording allowed the determination of the effect of prostigmine on the amount of activity present in the bowel, the types of contractions, their rate, duration and amplitude and the co-ordination between contractions by adjacent segments of the bowel. After the intramuscular injection of 0.5 mg. of prostigmine methyl sulfate an effect on the bowel was noted in from two to eight minutes. This consisted of a definite change of the character of bowel activity. There was a decrease in the number of *type 1* and *type 3* contractions and an increase in the number of *type 2* contractions. The average height of the *type 2* contractions increased and the incidence of co-ordinated *type 2* contractions rose. The proportion of each hour that the bowel was active was no greater after the drug than before. Thus prostigmine shifted motility toward propulsion which was indicated not only in the recordings but also by the expulsion of feces and gas.

*Local factors influencing deep rectal temperatures in man.*

JERE MEAD AND C. LAWRENCE BOMMARITO (introduced by H. S. BELDING). Quartermaster Climatic Research Laboratory, Lawrence, Mass

In experiments in this Laboratory in which rectal temperatures of men were studied, temperatures recorded at the tip of a 6-inch flexible rectal catheter have been as much as 1°F. lower than temperatures recorded immediately thereafter as shallower insertions. Studies have been conducted in an attempt to elucidate this seemingly paradoxical phenomenon and indicate its influence on rectal temperature as an index of internal body temperature. In 8 individuals studied under a variety of conditions of heat balance and imbalance, temperatures near the tip of a flexible catheter inserted 8 inches through the external anal sphincter were, in almost every instance, lower than temperatures recorded at intermediate points along the catheter. Deviations observed varied from 0.1° to 1.5°F. and were greatest in individuals whose body temperature was falling relatively rapidly (e.g. 2.5°F. in 20 minutes). X-ray films showed the tip of the catheter in a majority of instances to lie near the postero-lateral wall of the pelvis. Adjacent to the terminal portion of the catheter on the pelvic wall lies the hypogastric vein and its branches. These vessels carry blood passing from the surface of the buttocks, the upper legs and external genitalia. When these surfaces were subjected

to temperatures above body temperature the difference in temperature along the catheter was reduced to 0.1°F. When these surfaces were cooled below body temperature the temperature at the tip of the catheter dropped more rapidly than the other rectal temperatures. It was concluded that cooled venous blood was chiefly responsible for the deviations of temperature noted. Temperatures obtained anteriorly in the rectum near the midline should be sufficiently distant from the pelvic walls so as to be uninfluenced by cooled venous blood returning from the surface of the body.

*Inhibition of brain dehydrogenases by anticholinesterases*

M. MICHAELIS (by invitation), N. I. ARANGO (by invitation) AND R. W. GERARD. Dept. of Physiology, Univ. of Chicago, Chicago, Ill.

The pharmacological actions of drugs with a known anticholinesterase activity are commonly attributed to their inhibition of cholinesterase. That effects on function need not parallel this inhibition and that other actions occur have been found by several workers. Yet the report (*Biochem. J.*, 42: 96, 1948) that, of over a dozen purified enzymes tested *in vitro*, only esterases were inhibited by fluorophosphonates has seemed to confirm this limited and specific action. It is often found, however, that inhibitions are lost as systems are purified; even the fluoroacetates, recognized as inhibitors of oxidations rather than of esterase, are inactive on oxidizing enzymes *in vitro* (*J. Biol. Chem.*, 170: 67, 1947). Moreover, other work from this laboratory has demonstrated an inhibition of nerve and brain respiration by anticholinesterases at physiologically effective concentrations. We have, accordingly, examined the action of DFP (di-isopropyl-fluorophosphate), TEP (tetraethylpyrophosphate), and ES (eserine sulphate), all powerful anticholinesterases, and of MFA (methylfluoroacetate), a metabolic inhibitor, on dehydrogenase activity of brain and nerve. The Thunberg methylene blue reduction technique was used and decoloration times obtained for tissue homogenate blanks, tissue plus each of eleven substrates, and tissue-substrate plus each of the inhibitors at concentrations from  $10^{-4}$  to  $2 \times 10^{-2}$  M. Even at the lowest concentration, DFP inhibited the oxidation of succinate, lactate or glucose by about 15%, and TEP inhibited glucose by 25%, while MFA inhibited glucose, citrate or malate 10 to 20%. At  $10^{-3}$  M, DFP inhibited all dehydrogenases, from 90% for glucose, to 5% for citrate; TEP inhibited all but two, succinate and glutamate, glucose being most affected, with 60% inhibition; and MFA inhibition was greatest on malate, 30%, and zero for lactate. ES was inhibitory only at concentrations above  $2 \times 10^{-3}$ , glucose being again most sensitive. The anticholinesterase drugs thus possess

other actions which must be considered in explaining their pharmacology. DFP and TEP are at least as effective in inhibiting brain dehydrogenases as is the non-antiesterase, MFA.

*Resistance of explanted gastric mucosa to various chemical and physical agents.* JOHN REGIS MILLER, J. F. HERRICK, FRANK C. MANN, JOHN H. GRINDLAY, AND JAMES T. PRIESTLY. Divs. of Experimental Medicine and Surgery, Mayo Foundation and Clinic, Rochester, Minn.

Many methods have been devised to study the gastric mucosa and its secretions since Beaumont reported his classic observations in 1833. In order to study the local effects of various chemical and physical agents on the gastric mucosa, sizeable portions of the fundi of dogs' stomachs were explanted to the abdominal wall. The presence of acid on these vagotomized explants in response to a meal or to histamine stimulation was used as a test of function. Zinc chloride, tannic acid, sodium morrhuate, oil of peppermint, protamine, water soluble vitamin K, quinone and various pituitary preparations were applied directly to the mucosa by means of gauze sponges. The effects of various physical agents including hyperthermia, microwave diathermy, ultrasonics and ultraviolet irradiation on the function of the parietal cells of the explanted mucosa were noted. Gross observations were recorded and when indicated biopsies were taken and microscopic sections examined. Strong escharotics produced widespread destruction of the mucosa, but progressive return of function over a one-year period was observed. The mucosa was found to recover rapidly from the effects of local vasoconstriction, local heating, ultraviolet and bodily temperatures ranging as high as 108°. Ultrasonics caused a temporary suppression of the secretion of the parietal cells.

*Clinical and experimental aspects of the use of fluorescein dyes to diagnose central nervous system tumors.* GEORGE E. MOORE. Dept. of Surgery, Univ. of Minnesota, Minneapolis.

Fluorescein, a highly fluorescent acid chromagen dye, has been used to help differentiate normal and malignant tissues. The most consistent results have been obtained in the examination of brain tumors. Tumor tissue secured from suspected areas by aspiration needle biopsies are readily recognized by the exaggerated fluorescence observed under ultra-violet light. Both glioma and meningioma groups of brain tumors have been recognized correctly as tumor tissue. The expediency and efficiency of this method is self evident: fewer microscopic sections are required, the time saved is considerable, and the neurosurgeon can check all parts of the needle biopsy for tumor. To date this

procedure has been used in 65 cases; two errors in diagnosis have been made. More recently various iodine substitution products of fluorescein have been synthesized. Attempts to use radioopaque dye (tetraiodophthalic fluorescein) for the direct localization of brain tumors by roentgenography have not been uniformly encouraging. Radioactive diiodofluorescein (I<sub>131</sub>) has been employed in an effort to diagnose and localize brain tumors before operation. Dye containing approximately 1 millicurie of activity is injected intravenously and following a 2-hour interval, counts are taken over symmetrical areas of the head by means of a specially shielded Geiger-Mueller counter. The source of greatest activity can then be triangulated. Tumors less than 3 cm. in diameter cannot be detected by our present methods unless they are superficial and surrounded by an appreciable amount of edema. In addition small tumors situated at the base of the brain and near the midline are difficult to localize. The reason for the differential predilection of central nervous system lesions for fluorescein dyes is not at all clear. The so-called blood-brain-barrier plays a part, if not the major role, in the process.

*Coagulation time of whole blood as measured in silicone coated tubes before and after various surgical procedures.*

J. L. MORGAN (by invitation), NELSON W. BARKER (by invitation) AND GRACE M. ROTH. Sections of Internal Medicine and Physiology, Mayo Foundation and Clinic, Rochester, Minn.

An objective method for determining the coagulation time of whole venous blood was designed combining the use of silicone coated tubes with the coagulochronometer as described by Barker and Barker (*Proc. Staff Meet. Mayo Clinic*, 23: 230-233, 1948). The tubes used were screw cap glass vials sealed by bakelite caps and were designed by the Kimble Glass Co. Syringes, needles and tubes were coated with General Electric 'Dri Film 9987'. The mean value for the coagulation time of whole blood as determined by this method employing fifty-one normal subjects was twenty-five minutes. Twenty-five patients were studied by coagulation time determinations done on the day before operation, and the second, fourth, sixth, and eighth days after various surgical procedures. No uniform changes were noted in the coagulation times of these patients on the different days after the surgical procedures. One patient died of a massive pulmonary embolus on the seventh postoperative day and no change was noted in the coagulation time of the blood of this patient. Therefore, by this method, which incorporates a prolonged normal value of the coagulation time of whole blood, no uniform changes of the coagulation time could be detected after surgical procedure and no pre-

dictions of impending venous thrombosis could be made.

*Nitrogen balance index and specific dynamic action in rats receiving amino acid mixtures low in isoleucine, methionine or valine.* E. S. NASSET AND JOSEPH T. ANDERSON. Dept. of Physiology and Vital Economics, Univ. of Rochester, Rochester, N. Y.

N balance and energy metabolism were determined simultaneously on adult rats fed amino acid mixtures. When a 'complete' amino acid mixture, simulating egg protein, was fed after a 7-day 'N-free' diet period, the N balance index was invariably greater than unity. Reducing the DL-isoleucine to  $\frac{1}{2}$  resulted in a significant decrease in N balance index and an increase in SDA. A similar reduction in DL-methionine halved the N balance index without affecting the SDA. Neither the reduction of DL-valine to  $\frac{1}{2}$  nor the substitution of glycine for glutamic acid in the mixture caused any significant change in N balance index or SDA. The total N requirement for equilibrium on the complete amino acid mixture was computed to be 160 mg. N/day/kg<sup>0.75</sup>. Amino acid requirements were: 1) 6.5 mg. DL-isoleucine N/day/kg<sup>0.75</sup>; 2) 4.9 mg. DL-methionine N/day/kg<sup>0.75</sup>; 3) and less than 5.9 mg. DL-valine N/day/kg<sup>0.75</sup>.

*Effect of topically and systemically administered pilocarpine on the denervated iris of the cat.* ERIC A. NEEDLE (introduced by WALTER S. ROOT). Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.

Twenty-five cats, subjected to unilateral removal of the ciliary ganglion, were used to demonstrate the 'sensitization' phenomenon ('paradoxical pupil constriction') to pilocarpine. The drug was either applied topically into each conjunctival sac at 0.04 mgm. or administered systemically into the femoral vein at 0.4 mg/kg. of body weight. These doses were chosen because the denervated pupil responds by maximal constriction to these doses whereas the normal pupil, which served as a control, showed no response. Certain differences have been observed in the reactions of the denervated iris to topical and systemic administration of pilocarpine and these are briefly: 1) the latent period for the action of pilocarpine given topically is longer than for intravenous administration; 2) maximal sensitization to topical pilocarpine follows immediately after operation while maximal sensitization to intravenous pilocarpine develops six to eight days following denervation; 3) the response of the denervated iris to topical pilocarpine is at all times more intense and long-lasting than is its response to intravenous pilocarpine; 4) the response of the denervated pupil to topical pilocarpine is not significantly altered either with the passage

of time or as a result of repeated doses of the drug, whereas, in the same animal, the response to intravenous pilocarpine appears to decrease with repeated dosage. This finding probably cannot be explained on the basis of possible regeneration to the sphincter since in only three of the 25 cats in the series was there any decrease in size of the formerly maximally dilated pupil or any return of light reflex. In addition, the decreased response of the pupil after repeated administration of the drug does not seem to be related to time but rather to the number of previous intravenous doses of the drug.

*Regulation of capillary flow in subcutaneous tissue.*

PAUL A. NICOLL AND RICHARD L. WEBB (by invitation). Depts. of Physiology and Anatomy, Indiana Univ., Bloomington.

The actual characteristics of blood flow through capillaries can only be determined by direct observation of these vessels in a suitable field. In the mammals such access are indeed limited. The observations here reported were made on the thin membrane of the bat's wing. Technical details have been reported elsewhere. It is only necessary to emphasize that the preparation allows one to study normal capillary beds in their normal environment. Assuming an adequate pressure gradient from artery to vein, flow behavior in a given capillary is chiefly determined by three factors: First and most important is the nature of the active vasomotion exhibited by the supplying arteriole and precapillary sphincter. This of course determined the effectual pressure head at the capillary origin. Secondly, the types of interaction between the endothelial tubes and the formed elements of the blood determine largely the magnitude of the capillary resistance. The third factor is the venule behavior which regulates the flow out from the capillaries. Active vasomotion is the result of contraction or relaxation of spirally wound smooth muscle cells along the arterioles and terminal arterioles. Their behavior appears regulated by a dynamic equilibrium between the cells and their interstitial environment. No direct nerve connections can be demonstrated, although impulses passing along adjacent fibers do effect their behavior to some extent. The main regulation appears to depend on changes in metabolites in the cells immediate environment. Although each cell is an independent effector the usual condition is a rhythmical contraction and relaxation along an entire terminal arteriole. This produces alternate flow and quiescence in the capillary beyond. It is suggested that this behavior allows alternating periods of outward and inward movement of water and dissolved substances along the entire capillary bed. Thus during the period of constriction the hydrodynamic pressure factor within

the lumin would fall to zero and the only affective force active would be the solvent diffusion pressure (i.e. colloidal osmotic pressure) from interstitial space to capillary.

*Protein pattern of liver and intestinal lymph.* JAMES T. NIX. Mayo Foundation, Rochester, Minn.

Grindlay and Cain (1947) developed a simple, reliable method of intubating lymphatics with polythene tubing that has made possible a broader investigation of the protein moiety of liver and intestinal lymph. Accordingly, the object of this investigation was to determine average values for the concentration of protein fractions and the rate of protein flow of liver and intestinal lymph in the normal dog under constant experimental conditions. Observations were made on specimens from 13 liver lymphatic cannulations and 10 intestinal lymphatic cannulations. Average values for pure liver and intestinal lymph in the normal dog were controlled by blood studies; by comparison with mixed liver and intestinal lymph from the thoracic duct of the normal dog; by contrast with pure and with mixed liver and intestinal lymph from dogs with liver disease, experimentally produced; by supplemental studies on cisternal lymph from the albino rat. Protein was fractionated by sodium sulfite precipitation; the concentration of protein fractions was measured by Kjeldahl technic.

*Time of appearance of flare and wheal after intracutaneous histamine.* ERIC OGDEN, P. A. CLOUSE, AND R. V. MURRAY, JR. Dept. of Physiology, Univ. of Texas Medical Branch, Galveston, and Scott and White Clinic, Temple, Texas.

Using the multiple puncture technique of intradermal administration of 1:1000 histamine, the authors undertook to determine what measurements of the histamine reaction would be most reliable and to establish normal values for these measurements in subjects with no evidence of vascular disease. Attempts to measure the response by the size of the flare or wheal were abandoned as unreliable, and final correlation of measurements was limited to the interval between the puncture and the appearance of the response. Seventy subjects were tested, ranging in age from 14 to 76. Five test sites were used: the brow, epigastrium, volar aspect of the forearm, lateral aspect of the ankle and the dorsum of the foot. Results were as follows: the average times for the flare on the brow, epigastrium, and forearm were 21, 25, and 30 seconds, whereas on the ankle and foot the times were 55 and 61 seconds respectively. The average times for the appearance of the wheal were 99, 91, and 96 seconds for the brow, epigastrium, and forearm respectively, and for the ankle and foot 168 and 159 seconds. There was no

significant relationship shown between onset of the flare or wheal and age. There was no correlation between the rapidity of onset of the flare and wheal and skin temperatures. The ankle and foot showed a slower reaction time than the areas on the upper portion of the body—approximately twice as long. This correlates with the findings of other investigators regarding reactive hyperemia in the forearm and foot.

*Variations in the oxygen tension and pH of renal cortex.*

NORMAN S. OLSEN AND HENRY A. SCHROEDER. Depts. of Biological Chemistry and Internal Medicine and the Oscar Johnson Institute, Washington Univ. of Medicine, St. Louis, Mo.

Changes in oxygen tension and pH of renal cortex of nembutalized dogs have been measure *in vivo*. Oxygen tension was followed by the macro-electrode of McCulloch and pH by a modified glass electrode. Both kidneys were exteriorized and an adjustable clamp placed around the left renal artery. A pH and oxygen electrode were placed on the surface of each kidney and changes recorded by means of a D.C. amplifier and inkwriter. On slight constriction of the artery, the left kidney showed a decrease in oxygen tension to about 20% of the control value and a decrease of about 0.2 of a pH unit. This was transient, returning to the control level in about 15 minutes, while the clamp remained tightened. Essentially no changes were noted in the opposite kidney. Complete occlusion of the artery lowered the oxygen tension of that kidney to practically the anoxic state concomitant with a decrease of over 0.5 of a pH unit. In the contralateral kidney there seemed to be a slight significant rise in oxygen tension and no change in pH. These changes remained at their altered levels until the clamp was released, whereupon they returned to their former values. Varying degrees of constriction gave intermediate results. The intravenous injection of 100 of epinephrine temporarily decreased the oxygen tension of the renal cortex. By constricting the left renal artery the epinephrine effect was lessened in that kidney. On complete occlusion no change in oxygen tension could be elicited by this amount of epinephrine. The response of the opposite unstricted kidney to the drug was not altered by any degree of clamping.

*Effect of intravenous procaine on the heart.* M. J. OPPENHEIMER, JOAN H. LONG, MARY R. WESTER, THOMAS M. DURANT. Temple Univ. School of Medicine, Philadelphia, Pa.

Single therapeutic intravenous doses of procaine (4 mg/kg.) usually produce no change in the electrocardiogram, heart sounds and blood pressure in anesthetized dogs. An increase in *T* was found occasionally. At 8-10 mg/kg. *T* was consistently

changed, usually higher, accompanied by lower voltage in R, increased voltage in S, with formation of 'J' in precordial leads. At 20 mg/kg. QRS was wider, increasing five-fold in width up to 60 mg/kg. Doses of 30 mg/kg. increased PR intervals. At 50-60 mg/kg. ventricular tachycardia resulted. Provided artificial respiration was maintained the above changes were reversible even though blood pressure was very low. At 60-80 mg/kg. ventricular tachycardia, flutter and fibrillation appeared in sequence and artificial respiration was of no avail. One dog with an abnormal heart fibrillated at a much lower dose. Blood pressure, pulse pressure and heart sounds are decreased as QRS widens. This indicates a decreased force of contraction. Spontaneous respiration ceased at 30-40 mg/kg. Serum potassium was unchanged at all doses. The extensive intraventricular block with high doses should be stressed in relation to preexisting arrhythmias and subsequent ventricular fibrillation.

*Factors related to voluntary ventilation capacity.* A. B. OTIS AND W. C. BEMBOWER (by invitation). Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.

The maximum voluntary breathing frequency for 15 seconds was determined at various tidal volumes in each of 5 subjects. The data are reasonably well described by the equation,  $T = bV_T + T_0$ , where  $T$  = the time for one cycle;  $V_T$  = the tidal volume;  $b$  and  $T_0$  are constants. The minute volume is consequently given by  $V_M = V_T/b V_T + T_0$ . This indicates that maximum ventilation is performed with the largest possible tidal, but the constants are such that there is little gain with tidals greater than 0.3 the vital capacity. The value of  $T_0$  is about 0.08 seconds and may represent the maximum time required for the neuro-muscular mechanism involved to alternate. These equations imply that the mean velocity during a cycle is independent of the tidal volume, but this has not been determined experimentally by a study of breathing patterns. When the above tests were performed using 80% He - 20% O<sub>2</sub> as the respired gas,  $T_0$  remained practically unchanged but  $b$  was diminished and the maximum ventilation was larger. The resistance of the respiratory passages to gas flow is therefore a factor determining voluntary ventilation capacity. This has been confirmed by simultaneous measurements of alveolar pressure and velocity of flow in 29 subjects for whom the voluntary ventilation capacity and the vital capacity were known. The alveolar pressure necessary to produce a flow of 1 l/sec. was taken as a measure of resistance.  $V.V.C.$  showed a negative correlation with resistance ( $r = -0.57$ ) and a positive correlation with vital capacity ( $r = +0.79$ ).

*Vascular responses to temperature in the isolated perfused hindlimb of the cat.* J. R. PAPPENHEIMER, S. L. EVERSOLE, JR. (by invitation) AND A. SOTO-RIVERA (by invitation). Dept. of Physiology, Harvard Medical School, Boston, Mass.

The temperature of arterial blood supplying the perfused hindlimb of the cat was varied over the range 40 to 5°C. As the arterial blood is cooled from 40 to 25°C. the blood flow at constant pressure is diminished. Below about 25°C. however, the flow increases progressively; at 5-10°C. the blood flow generally exceeds that at 40°C. If the blood vessels are poisoned with NaCN, these vascular responses to temperature are abolished; in the cyanided limb the blood flow diminishes with temperature in proportion to the diminished fluidity of the blood. Evidence will be given that the vessels in the paw and skin respond to cold by vasoconstriction whereas the muscle vessels progressively dilate as the temperature is reduced. At 40°C. the paw accounts for about 30% of the total flow through the limb whereas at 25°C. it accounts for less than 5% of the total flow. Air injected into the femoral artery normally appears in the saphenous vein in the form of large bubbles. However, if the paw is excluded from the circulation or if the blood is cooled, then the intra-arterial injection of air effectively blocks the circulation. Evidently flow in the paw is through large blood vessels, presumably arterio-venous anastomoses, which constrict when exposed to cold blood. The direct constrictor effect of cold on the blood vessels of the superficial tissues (in contrast to the dilator action of cold on the blood vessels of the deep tissues) is of obvious importance to the control of body temperature.

*Studies of the anti-heparin compound, protamine.* THOMAS W. PARKIN AND HIRAM E. ESSEX. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

It has been known since 1938 that certain protamines will neutralize heparin *in vitro* and in animals. At the time this investigation was started, there were no reports in the American literature concerning the clinical use of protamine for the neutralization of heparin. The purpose of this investigation was to determine by animal studies the toxicity of the protamine, salmine. It is of interest to note that protamines have been proposed recently for the treatment of hemorrhage occurring in certain cases of thrombocytopenic purpura. The intravenous injection of the protamine, salmine, produced toxic effects in the guinea pig, rabbit and dog when large doses were used. The principal toxic effects in the guinea pig were the result of broncho-



constriction and the lethal dose was 6.0 mg/100 gm. of body weight. The principal toxic effect in the rabbit and dog was a fall in the arterial blood pressure. Intravenous injection of salmine also produced constriction of the blood vessels in the ear of the rabbit; this effect was observed through a transparent window placed in the ear. Concomitant with the fall in arterial blood pressure which occurred in the dog after the intravenous injection of salmine, an increase in the portal venous pressure was observed. The fall in the arterial blood pressure which occurred in the rabbit and dog was prevented by the intravenous injection of diphenhydramine (benadryl) shortly before the protamine was injected. This evidence suggests the possibility that release of histamine may be a factor responsible in the production of toxic effects following the intravenous injection of salmine. Intravenous injection of salmine promptly neutralized the anticoagulant effect produced by intravenous injection of heparin in the dog. The anticoagulant effect produced by the intramuscular injection of heparin in Pitkin's menstruum in the dog was temporarily abolished by the intravenous injection of salmine.

*Changes in heart rate, blood pressure and electrocardiogram in dogs during diffusion respiration.* THOMAS M. PARRY (by invitation), JOSEPH N. SPENCER (by invitation), RICHARD W. WHITEHEAD AND WILLIAM B. DRAPER. Dept. of Physiology and Pharmacology, Univ. of Colorado Medical Center.

Fifteen experiments were conducted in which the changes in the heart rate, blood pressure and electrocardiogram were observed in dogs during diffusion respiration. In addition to a severe respiratory acidosis, the circulation during diffusion respiration is subjected to other abnormal and presumably injurious influences, viz.: an overdose of pentothal sodium, the absence of the respiratory movements and of the 'pulmonary-vascular pump', a high degree of blood concentration and, from the 30th minute onwards, a progressively increasing hypoxemia. Following a 45-minute period of denitrogenation apnea was produced and maintained by the administration of an overdose of 1% pentothal sodium (11 expts.) or by a combination of intocostirin and pentothal sodium (4 expts.). At intervals during the experiment the pH of the abdominal arterial blood was determined and electrocardiogram tracings obtained. The femoral arterial blood pressure was continuously recorded by means of a mercury manometer. The establishment of apnea was not accompanied by significant changes in the heart rate. The systolic blood pressure during diffusion respiration showed an initial fall but thereafter was

well maintained during the period of respiratory arrest. The electrocardiogram revealed the presence of ventricular extrasystoles during the first phase of diffusion respiration which disappeared during the latter phase of apnea. Although diffusion respiration proceeded satisfactorily when a combination of pentothal sodium and intocostirin was used to produce apnea the more severe initial drop in blood pressure and greater difficulty in resuscitation makes its use for this purpose inadvisable.

*Analysis of skin, muscle and brachial arterial blood temperatures in the resting normal human forearm.*

HARRY H. PENNES. Psychiatric Institute of New York, New York City.

Temperatures were measured by radiometric and thermocouple techniques in 64 basal, almost nude male and female subjects prone on a hospital bed, the right forearm supported in air. Identical air and wall temperatures ranged from 25.0–27.5°C.; air relative humidities from 45–75%, with linear air velocity below 20 feet per second. In 10 subjects brachial arterial blood temperatures (average 36.68°) were 0.00 to 0.36° higher than maximum deep forearm temperature (average 36.52°). Skin temperature gradients on the superior surface of the longitudinal axis of the forearm averaged 0.03° per cm. in 14 subjects. Gradients in 17 subjects around the forearm circumference were highly irregular; average maximum difference between any two forearm points was 1.2° for the group. Temperature curves through the complete transverse forearm axis were approximately parabolic. Lack of perfect circular symmetry of the curves precluded individual logarithmic analysis. Theoretical thermal distributions in solid tissue cylinders were plotted by combining the general differential equation of heat flow in homogeneous, isotropic conductors, the Fick principle of physiology, and the Newton Cooling Law. Principal assumptions made were uniformity of rate of tissue heat production, volume flow of blood, and thermal equilibration constant between capillary blood and tissue. Closest approximation of the mean experimental tissue temperature curve was obtained with substituted values of local heat production of 0.0001 and blood flow of 0.0002–0.0005 c.g.s. units, both of which values agree closely with experimental data reported by other workers.

*An improved method for recording capacitance changes accompanying the cardiac cycle.* EDWARD PERL (by invitation) AND WILLIAM V. WHITEHORN. Dept. of Physiology, Univ. of Illinois, College of Medicine, Chicago.



The use of capacitative changes in a condenser whose field contained the heart to study variations of cardiac volume was first suggested by Atzler and Lehmann (*Arbeitsphysiol.*, 5:636, 1932). Capacity of such a

condenser is given by:  $C = 0.0885 \frac{AK}{K(D-T) + T}$

where  $A$  = area of plates,  $K$  = dielectric constant of the chest,  $D$  = distance between plates and  $T$  = thickness of the chest. Varying amounts of blood in the heart and vessels in the field during the cardiac cycle change  $K$ , thus altering capacity. Difficulties in previous methods of measuring these changes were primarily in adjustment and lack of stability and linearity of response. We have employed newer technics to circumvent these obstacles. The condenser was incorporated in the frequency determining circuit of an oscillator. Its output was passed through a broadband amplifier and a limiter circuit and fed to a discriminator which converted the change in frequency to a D. C. voltage which linearly represented capacitative variations. This was amplified and recorded on a string galvanometer. An oscillator frequency of 10.7 megacycles was chosen to obtain simplicity of circuits, large output and minimum loss current through the poor dielectric of the chest. The apparatus is simple in adjustment and operation and gives consistent, reproducible and linear records in both model and human experiments.

*Cerebral projections to cerebellar nuclei with special reference to area 4S.* ERIC W. PETERSON, RAY S. SNIDER AND WARREN S. MCCULLOCH. Dept. of Psychiatry, Univ. of Illinois School of Medicine, and Dept. of Anatomy, Northwestern Univ. Medical School, Chicago.

By local applications of strychnine to several cortical areas and enough pick-up electrodes on the cortex to define the areas strychninized, the depths of the cerebellum were grouped with bipolar electrodes in a stereotactic instrument and the positions of recordings in it verified histologically. The results indicate that there is a strong projection from areas 4s and perhaps 6 to the homolateral as well as a weaker projection to the contralateral nuclei. If there is a projection from area 4 it resembles that via the motor horn cells to the muscles, for strychnine spikes to the cerebellar nuclei are evoked from 4 only when twitches in the muscles are present.

*Intra-arterial changes in pressure before, during, and after exercise.* L. H. PETERSON, T. G. SCHINABEL, JR., H. FITZPATRICK AND H. C. BAZETT. Dept. of Physiology, Univ. of Pennsylvania, Philadelphia. Continuous direct intra-arterial pressures have been recorded during strenuous exercise of four types at four

work levels. Recording is done through a flexible catheter in the brachial artery, capacitance manometer and ink writer. Fifteen subjects performed step tests (including one athlete) and in addition 4 subjects performed bicycle tests (including a national bicycle sprint and distance champion). The  $\Delta$  value of the product of pulse pressure and pulse rate (rest to exercise) was used as an index of changes in cardiac output in any single subject. Non-athletes show a rise in apparent cardiac output in proportion to the intensity of work. The athlete's circulation increases to a slightly greater degree than the non-athlete's doing step tests and a markedly greater increase when doing the bicycle tests. His diastolic pressure rises appreciably during the step test, but does not rise while riding a bicycle. It appears that the athlete shows an increase in cardiac output which is disproportionately large, with evidence of more effective vasodilatation when doing work for which he is trained. All subjects show a fall in total resistance while exercising. When exercise ceases there is usually a marked, immediate drop in mean pressure with little or no reduction in pulse pressure or pulse rate. This suggests that the simultaneous opening of some additional vascular bed offsets the fall in venous return which would result from the loss of the muscle pump, etc.  $\text{CO}_2$  inhalation abolishes this immediate drop. The drop is followed by undulations of the blood pressure during which time the pressure levels and pulse rate are markedly affected by body movements.

*Hyperglycemic activity of pancreatic extracts.* I. J. PINCUS, S. A. KOMAROV, W. J. SNAPE AND H. SHAY. Samuel S. Fels Research Institute, Temple Univ. Medical School, Philadelphia, Pa.

Highly purified as well as relatively crude extracts of pancreatic tissue containing insulin are known to elevate blood sugar so as to partially obscure or counteract the insulin activity. Extracts prepared from the pancreas of dogs previously treated with alloxan produce a more marked elevation suggesting that the beta cell does not elaborate this factor. Similar preparations of dog and steer parotid glands fail to provoke hyperglycemia. Our studies on insulin inactivated by the method suggested by Southerland and Cori revealed that intravenous injection of large amounts of inactivated insulin produces a more marked and more sustained rise in blood sugar levels than is provoked by unmodified insulin preparations. Somewhat less of a response is noted after the intraperitoneal injection of either of these preparations with, however, a considerable delay usually present before the peak is reached. Subcutaneous injection minimizes the hyperglycemic activity. Inactivated insulin produces a hyperglycemia in the pancreatectomized as well as the

normal animal; however after hepatectomy and enterectomy only a continued fall in blood sugar was observed. NOVO insulin contains only small amounts of the hyperglycemic factor. Intravenous injection of very large doses of this preparation produces rises in blood sugar of very short duration and inactivation destroys the small amount of hyperglycemic factor present.

*Effect of exercise and body position on the venous pressure at the ankle in patients with varicose veins.* ALBERT A. POLLACK (by invitation), BOWEN E. TAYLOR (by invitation), EARL H. WOOD AND THOMAS T. MYERS (by invitation). Mayo Foundation, Rochester, Minn.

Direct and continuous measurements of the pressure in the great saphenous vein at the ankle have been made in 13 patients with incompetent veins of the lower extremity when in the supine, sitting and standing positions, and during walking on a treadmill. Pressures were determined by means of a strain-gauge manometer connected to a polythene catheter inserted into the vein through a 17 ga. needle. The average pressure with the patient in the supine position was 8.5 (5.7 to 14) mm. Hg; in the sitting position, 52 (31 to 61) mm. Hg; and in the resting, standing position, 81 (63 to 89) mm. Resting venous pressure at the ankle with the patient in these positions was sufficient to support a column of blood up to the third thoracic interspace at the sternum. This was also found to be true in normal subjects. In 7 patients who had uncomplicated venous insufficiency of the superficial system, walking at 1.7 miles/hr. uniformly produced a decrease in the venous pressure at the ankle, during the first 3 to 6 steps, to an average mean stable value of 44 (34 to 56) mm. Hg which was maintained for the duration of the thirty to sixty second period of walking. In normal subjects similar exercise decreased the venous pressure at the ankle during the first 4 to 12 steps to a mean stable value of 22 (11 to 31) mm. In the patients with incompetent superficial veins, the venous pressure at ankle returned to the resting standing level within 2.8 (1.2 to 5.5) seconds after the walk was completed, as contrasted with the 31 (8 to 57) seconds required in normal subjects. In 6 patients with a history of iliofemoral thrombophlebitis in addition to incompetency of the great saphenous veins the pressure decreased during the first three to five steps to an average mean stable value of 76.5 (47 to 96) mm., and increased to an average value of 3 (1 to 8) mm. higher than the resting standing control value within one second after completion of the walk.

*Healing of experimental wounds of the lung.* C. B. PORTER, G. M. HIGGINS, AND O. T. CLAGETT, Mayo Foundation, Rochester, Minn.

Experimental studies were made to determine the mode of healing of mammalian lung tissue after surgical injury. The present paper covers studies made on the lungs of the albino rat, the rabbit and the dog. In the rat, simple incisions were made in the anterior lung edge and were allowed to heal without the use of sutures. A second series of incisions in the rat lung were sutured and allowed to heal. Vital staining was used to delineate the phagocytic cells concerned in lung repair in the rat. In rabbits and dogs small wedges of lung tissue were excised, the defects closed with fine sutures, and the healing process studied. In the rat and dog, healing occurred with the formation of a connective tissue scar similar to scars elsewhere in the body. No regenerative phenomena were noted. In the rabbit fibrous healing also occurred but was accompanied by abortive regenerative phenomena on the part of the injured bronchial tree. The end result however was a fibrous scar containing no new formed pulmonary tissue.

*Increase in irritability of a mammalian sensory nerve following ischemia.* E. L. PORTER AND J. L. COLEMAN (by invitation). Dept. of Physiology, Univ. of Texas School of Medicine, Galveston.

Wharton and Porter (*Federation Proc.* 6: 181, 1947, and in press, *Journal of Neurophysiology*) have reported a method by which a segment of mammalian motor nerve (cat peroneal) can be rendered ischemic at will, and repeatedly, and the effects observed, using contractions of tibialis anticus muscle as indicator. The effect of ischemia for the first few minutes was a marked increase in irritability as indicated by higher contractions of the muscle. It has been found possible to repeat this procedure on a sensory nerve (posterior tibial of cat). Reflex contractions of tibialis anticus muscle indicate changes in irritability of the nerve during and after ischemia. These contractions increase in height following the clamping of the blood vessels. Removal of the clamp after 2-3 minutes results in a decrease in reflex contraction height to the original level within the following few minutes. The experiment may then be repeated. If the clamp be left on the vessels, the contraction heights remain high for some 10 minutes and then diminish and disappear during another 10 minutes. An interval of a half hour or more is then necessary before the experiment can be repeated. The results indicate that a sensory nerve behaves toward ischemia just as the motor nerve does. The reflex contraction of tibialis anticus is a part of the flexion reflex. In the acute spinal cat this reflex can only be elicited by stimuli which would be painful to the conscious animals. Our results, therefore, have a bearing on the question of ischemic pain as,

for example, that following the plugging of an artery by a thrombus.

*Effect of humidity on the change in body temperature of the albino rat during exposure to low atmospheric pressures.* F. H. QUEMBY, N. E. PHILLIPS, B. B. CARY AND R. MORGAN. Dept. of Zoology and Physics, University of Maryland, College Park.

The body temperature of the rat at reduced pressures shows no change when the air supplied to the animal contains oxygen at sea level equivalent. However when employing normal air there is a significant reduction in body temperature with each drop in pressure. A definite relationship between the partial pressure of oxygen and the body temperature is shown by two factors: first at each reduced pressure the newly established body temperature, once adjusted, is maintained as long as that pressure prevails; and second, when the animal is returned over the same increments to normal pressure the body temperature at each pressure is remarkably close to those established at corresponding pressures maintained during pressure reduction. In moist air the temperature effect of pressure reduction is delayed and less severe. The average fall in body temperature at 260 mm. Hg pressure is 6.1°C. in dry air and 2.9°C. in moist air, the higher temperature in the case of the latter being due to the fact that in humid air the animal can lose little heat by evaporation from the respiratory and external surfaces. This heat saving effect may result in reduced energy metabolism and a decrease demand for oxygen, which in turn may account for the increased resistance which animals in moist air offer to anoxia.

*Inhomogeneity of alveolar air.* HERMANN RAHN. Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.

Individual alveoli or parts of the lung are neither equally ventilated nor circulated thus producing inhomogeneity of the alveolar gases. It is desirable to predict the relative O<sub>2</sub> and CO<sub>2</sub> concentrations that must exist for the infinite number of ventilation/blood-flow ratios in any part of the lung. By combining the alveolar ventilation equation (Fenn '46) with the Fick equation one finds an expression for the alveolar CO<sub>2</sub> or O<sub>2</sub> in terms of bloodflow and ventilation. Thus for example the

$$\text{alv. p CO}_2 = \frac{.864 \times \text{bloodflow} \times (V-A) \text{ CO}_2}{\text{alv. ventilation}}.$$

Assuming particular venous blood values it can be shown that each ventilation bloodflow ratio produces a definite alveolar respiratory quotient. Furthermore, all possible simultaneous alveolar O<sub>2</sub> and CO<sub>2</sub> values must fall upon a definite curve when the O<sub>2</sub> is plotted

against the CO<sub>2</sub>. Experimental values agree fairly well with the theoretical ones.

*Quantitation of the output of the sweat glands and their response to normal stimulation.* WALTER C. RANDALL AND WARREN MCCLURE (by invitation). Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.

Simultaneous observations upon the quantity of sweat produced and the number of functioning sweat glands on a given area provide data from which an estimation may be made of the quantity of sweat discharged by a 'typical' sweat gland in the area. It should be emphasized that the sweat glands are not continuously active but periodically discharge sweat upon the skin surface. During the normal sweating responses of a subject at rest in a warm environment, the average output of the glands of the arms and legs is from .0037 to .0043 mg/min., while that on the dorsal surfaces of the hand and foot is .002 to .003 mg/min. It is not known at present whether this apparent variation represents morphological or functional differences in the responses in these areas, but it is known that similar relationships exist in the larger outputs of the glands when stimulated directly by cholinergic drugs. The preliminary response of the sweating mechanism to the stimulation induced by mild exercise and by heat is to increase the number of functioning glands. If this response proves inadequate to meet the demands of temperature control, further evaporative heat loss may be brought about by an increased output of the individual glands over a given period of time.

*Effect of pyrimidines and thiopyrimidines upon liver, nucleic acids and regeneration.* DAVID RAPPORT, ATTILIO CANZANELLI, AND RUTH GUILD. Tufts College Medical School, Boston, Mass.

The background of this investigation was the possibility that administered pyrimidines might tend to increase the nucleic acid content of liver and its regenerative capacity, and that thiopyrimidines might reverse the effects by acting as competitive inhibitors. The relation to the thyroid was also studied. One per cent by weight of the various pyrimidines was added to the food of 3- to 6-month-old rats for periods of 10 or 20 days, following which  $\frac{1}{3}$  of the liver was removed. After 96 hours the animals were killed. Ribonucleic acid (PNA) and deoxyribonucleic acid (DNA) were determined by the method of Schmidt and Thannhauser, as well as total N and percentage regeneration. Wet wt./dry wt. ratio was also determined; it was unchanged under all the experimental conditions. The chief results were as follows: the administration

of uracil and thymine had no effect whatever. Thiouracil, thiothymine and propyl-thiouracil all reduced the regeneration, and after 10 days raised the total N, which after 20 days of thiopyrimidine returned to normal. Thiothymine reduced (after 20 days) both PNA and DNA; thiouracil and propyl-thiouracil had no effect on these. As we reported previously thyroidectomy resulted in a fall in PNA in the prelobectomized liver, and an equivocal fall in the percentage regeneration; while feeding dried thyroid in large doses (500 mg. daily) increased both as well as the DNA concentration. Neither thyroidectomy nor thiouracil changed this thyroid effect. However, when young animals were thyroidectomized, there was an unmistakable fall in the regeneration, and when dried thyroid was given only in approximately replacement amounts (3.4 mg. daily) the regeneration returned to normal, and PNA to above normal. Moreover, when thiouracil was given together with this amount of dried thyroid the regeneration was again reduced, though not the PNA. We concluded that the thyroid hormone is a regulator of liver regeneration, and that probably its function can be partly taken over in the absence of the gland. We also conclude that thiopyrimidines reduce the regenerative capacity of liver, thereby antagonizing the action of the thyroid hormone, probably by an effect on the enzymatic process in the liver itself. The postulated competitive inhibition of pyrimidines by thiopyrimidines was not proved, but is not ruled out, since nucleic acid pyrimidines may be synthesized in the body from smaller fragments.

*Electron microscope studies on tubercle bacilli (BCG) by shadow casting technique.* C. I. REED, B. P. REED (by invitation) AND SOL R. ROSENTHAL (by invitation). Depts. of Physiology, and Bacteriology and Public Health, Univ. of Illinois, Chicago Professional Colleges and Cook County Hospital, Chicago.

Chromium shadow casting of tubercle bacilli subjected to various techniques in the preparation of BCG vaccine were examined on the electron microscope. Artefacts were deliberately introduced in order to ascertain details of internal structure. Clear evidence of the existence of a capsule was obtained. Cytoplasm was usually collected in three or four masses suggesting that reproduction by cross-fission might be preceded by clumping of cytoplasm. Polar bodies could be seen in many organisms. The significance of these has not been determined. It is doubtful that branched bacilli exist. Surface contour was such as to indicate that distortion by drying is not a common phenomenon.

*Rapid removal of extracellular potassium.* ROGER M. REINECKE, CLEON R. HOLLAND (by invitation) AND FRANCIS L. STUTZMAN (by invitation). Dept. of Physiology, Univ. of Minnesota, Minneapolis.

By vivodialysis, using a modified Kolff dialyzer, it was possible to remove a quantity of potassium from the dog approximating or exceeding the total amount in the extracellular fluid within less than five and one-half hours. The animals survived this procedure.

*Effect of reduction of barometric pressure on the respiratory rates of acclimatized and unacclimatized rats.* O. E. REYNOLDS. Physiology Branch, Medical Sciences Division, Office of Naval Research, Washington, D. C.

Albino rats previously 'acclimatized' to anoxia by exposure to 18,000 ft. in a low-pressure chamber for 1 hr/day for 9 weeks and paired controls (10 rats/group) were exposed simultaneously to decreasing b.p. to a maximum simulated altitude of 36,000 ft. Respirations/min. (r/m) and extent of thoracic excursion were observed visually at 0 and 18, 25, 28, 32 and 36,000 ft. The average r/m of the acclimatized group was more rapid than that of the controls at sea level. The r/m of both groups decreased with increasing altitude, and the thoracic excursion increased concomitantly until the thoracic excursion became maximal (presumably when the tidal volume = vital capacity). After this point (25,000 ft. for controls; 28,500 for acclimatized) the r/m increased. The principal difference between the two groups consisted of a flattening of the r/m curve of the acclimatized group above 30,000 feet while the r/m for the control group was still increasing. If this difference is valid it indicates more rapid attainment of maximal ventilatory efficiency on the part of the acclimatized animals. These results are discussed with reference to human respiratory rate response to altitude.

*Pleural reaction to a polythene prosthesis after pneumonectomy in the rat.* JOHN RHYDELL, G. HIGGINS AND J. H. GRINDLAY. Institute for Experimental Medicine, Mayo Foundation, Rochester, Minn.

Left pneumonectomy was performed in a large series of rats; in some a lung-shaped polythene bag, filled with fluffed cotton and heat sealed, had been placed in the empty pleural space. It was found that there is a benign pleural reaction to such a prosthesis, and that the amount of exudate is not significantly increased over that seen after ordinary pneumonectomy. A neo-membrane forms about the polythene bag which gradually increases in thickness, reaching its maximum about one month after operation. In succeeding months, this contracts down to a thin transparent

fibrous rather acellular plaque. Use of the prosthesis effectively prevents shift of the mediastinum with resultant overdilatation of the remaining lung. Following ordinary pneumonectomy, the remaining lung rapidly increases in size and weight, so that by one month after operation it weighs almost as much as the normal combined weight of the two lungs. This increase in weight is largely prevented by use of the polythesis bag.

*Effects of rutin upon the capillaries with special reference to the bisulfite phenomenon.* R. K. RICHARDS AND KENNETH KUETER. Dept. of Pharmacology, Abbott Laboratories, North Chicago, Ill.

Recently considerable interest has been aroused by the effect of certain flavones upon pathologically increased capillary fragility in human beings. Attempts to demonstrate an effect of these substances upon the capillaries in experimental animals have met with considerable difficulties. The present investigations offer a new approach to this problem. It had been shown earlier (*J. Pharmacology* 79: 111, 1943, and *Anesthesia Analgesia*, 22: 283, 1943) that sodium bisulfite possesses a specific ability to increase the resorptive toxicity of epinephrine and procaine from subcutaneous and intramuscular sites if added in concentrations of 0.1 to 0.4%. This increase of toxicity, for which the name 'bisulfite phenomenon' is suggested, is due to an increase of the absorption rate produced by an effect of the sodium bisulfite upon the capillaries at the place of injection. The toxicity of epinephrine HCl by intramuscular injection in rats was markedly enhanced by addition of 0.1% sodium bisulfite. If however the rats were injected with 50 to 75 mg. solubilized rutin intravenously 10 minutes prior to the intramuscular injection of epinephrine the increase of toxicity caused by the sodium bisulfite could be considerably reduced. Such pretreatment with rutin was without effect upon the toxicity of epinephrine hydrochloride solutions as such. The increase of toxicity caused by the addition of sodium bisulfite to procaine hydrochloride solutions was likewise abolished by rutin pretreatment. However, rutin administration also reduced the toxicity of procaine hydrochloride alone. Procaine was shown to exert a dilating effect upon the capillaries which is counteracted by rutin. The conditions necessary for an increase of the toxicity of a drug by addition of sodium bisulfite (bisulfite phenomenon) are discussed and the action of rutin upon capillary permeability is viewed on the basis of these experiments which permit a quantitative approach for the evaluation of rutin-like compounds.

*Effect of tetraethylammonium chloride on gastric secretion in the dog.* C. R. ROBERTSON AND M. I. GROSSMAN.

Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

In the present study we have attempted to show the effect of tetraethylammonium chloride (TEAC) on gastric secretion stimulated by sham feeding, mechanical distention, urecholine, histamine and insulin in the dog. In 4 experiments the response to sham feeding was 66.6 mg. HCl/2 hr. When 10 mg/kg. of TEAC was given the response was 3.4 mg. HCl/2 hr. The TEAC presumably blocks the synapse of the pre- and post-ganglionic vagal fibers. In 15 control experiments with insulin the average gastric secretory response in 3 dogs with gastric fistulas was 67.8 mg. HCl/1.5 hr. The response in 13 experiments following the injection of 10 mg/kg. of TEAC was 13.8 mg. HCl. Here, also, the TEAC blocks the vagal ganglia. The gastric secretory response to distention with rubber balloons was decreased by TEAC in 9 out of 11 experiments on dogs with gastric pouches. The average control response in 11 tests was 28.7 mg. HCl/2 hrs. The average response after 10 mg/kg. of TEAC in 11 tests was 12.4 mg. HCl/2 hr. This decrease is due to the TEAC partially preventing the liberation of the gastric hormone in response to mechanical distention. In 6 tests on dogs with vagi intact and 10 tests on vagotomized dogs, histamine induced gastric secretion was not significantly decreased by 10 mg/kg. of TEAC. Urecholine induced gastric secretion was greatly reduced by TEAC in the dogs with intact vagi and reduced to a smaller extent in the vagotomized dogs.

*Various proteins and blood protein production.* F. S. ROBSCHT-ROBBINS. School of Medicine and Dentistry Univ. of Rochester, Rochester, N. Y.

The value for blood protein production of a variety of proteins has been ascertained in dogs rendered anemic and hypoproteinemic simultaneously. Considerable information has been obtained by determining the ratio of plasma protein output to hemoglobin output under these experimental conditions. The majority of proteins tested give values for such ratio below 50%. Egg albumen, lactalbumen, and fibrin among proteins so far tested behave differently. Supplements of some amino acids to egg albumen alter the picture.

*Some comparative aspects of the pulmonary arterial pressure.* S. ROBBARD AND F. BROWN. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago.

Measurements of the pulmonary arterial pressure in a reptile (turtle), mammal (dog), and bird (chicken) were used as a basis for a study of the comparative physiology of the lesser circuit. Data on the ventral aortic (pulmonary) pressure in the fish were available in the literature. In all these the pulmonary arterial pressure was

low, ranging around 25/10 mm. Hg. However, the systemic arterial pressure varied markedly from class to class. In the fish the systemic pressure was lower than that in the ventral aorta. In the turtle, the systolic pressures were equal in both circuits, but the systemic diastolic was higher than in the pulmonic; therefore the mean systemic pressure was also higher. In the homeotherms the systemic pressure was much higher than in the pulmonary circuit. The pulmonary arterial pressure remains low in the higher vertebrates probably as a result of factors which act to increase the rate of exchange of blood gases with those of the environment. These include: *a*) the enormous pulmonary vascular bed, *b*) the absence of tissue support of the pulmonary capillaries which are exposed over nearly their entire surface to the environment, resulting in *c*) great distensibility of the capillaries which *d*) provide the conditions for little increment in pressure with greatly increased volume. An increased pressure in such a capillary would produce a transudate and result in disturbed function. The complete division of the primitive ventricle into two chambers resulted not only in the separation of arterial from venous blood, but also provided conditions in which the systemic pressure could be raised without directly affecting the pulmonary blood pressure and blood flow.

*Histamine as the possible chemical mediator for cutaneous pain.* SOL ROY ROSENTHAL AND RALPH R. SONNENSCHIN. Univ. of Illinois College of Medicine, Chicago.

Previous studies have indicated that histamine, or a histamine-like substance, may be a peripheral mediator of cutaneous pain. By the use of sensitive biological methods, it was demonstrated that irritation of the skin or cornea by mechanical, electrical, or chemical stimuli, below the threshold for injury, is associated with the liberation of histamine or a histamine-like substance; the quantity liberated varies directly with the intensity of the stimulus. In addition, the perfusion of a solution of histamine onto the denuded skin or its intracutaneous injection is associated with painful sensations. As a corollary, it was shown that certain histamine antagonists (phenol ethers), in sufficient subcutaneous dosage, produced a generalized peripheral anesthesia in the dog, monkey, and human; on intracutaneous injection, they produce local anesthesia. It has since been reported that other anti-histamine drugs, of widely varying structure, likewise act as local anesthetics. The present report concerns itself with the determination of the minimum concentration of histamine necessary to produce sensations on intradermal injection. As a control, the actions of acetylcholine, potassium chloride, and adenosine, substances which might conceivably play a rôle in pain mediation were also tested,

alone and in combination with histamine. Twenty-seven adult subjects were tested. All chemicals used for injection were made up in 0.85% sodium chloride with distilled water. For control injections, the same salt solution was used. The subject was put at rest, was not told of the nature of the experiment or allowed to see the injections. A 22-gauge needle on a 2-ml. syringe was gently inserted as superficially as possible into the volar surface of the forearm. No injection was made until the pain from this prick had subsided. About 0.01 ml. was injected, producing a wheal of 2-3 mm. The injections were randomized with saline controls interspersed. There were as many saline placebo injections as of any given solution used. The subject was instructed to report immediately the occurrence of any sensation following the particular injection. This was noted and timed with a stop watch for a minimum of 3 minutes. In some 215 trials, it was found that painful sensations may be produced by the injection of histamine in concentrations as low as  $10^{-18}$  when introduced into the surface layer of the cutis. These findings are taken to indicate the specificity of histamine in the production of cutaneous pain, and to substantiate the postulate that a histamine-like substance acts as a physiological mediator of pain. Acetylcholine and adenosine apparently do not augment the cutaneous pain producing action of histamine. Potassium chloride in dilutions of 1:1000 or over gave no definite sensation. The fact that the production of itching requires a higher concentration of histamine than that necessary for pain indicates that itching is not a 'sub-threshold' pain. The least perceptible manifestations of pain are 'prickling,' 'stinging,' or 'tingling.'

*External pancreatic secretion in pancreatic fistula dogs.*

ERIC F. ROUTLEY, JESSE L. BOLLMAN AND JOHN H. GRINDLAY. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

The plan of this investigation is to study pancreatic juice (volume, amylase, lipase, trypsin, specific gravity and total protein) from chronic total pancreatic fistula dogs, before and after vagotomy and sympathectomy, under the following experimental conditions: *a*) fasting; *b*) various diets; *c*) sham feeding; *d*) ingestion of bile, alcohol, olive oil, and HCl; *e*) injection of secretin, mecholyl, histamine, adrenaline, insulin and pilocarpine. This preliminary report, based on some 300 specimens of pancreatic juice obtained from 18 pancreatic fistulae during the past nine months, lacks conclusive data. The 18 successful pancreatic fistulae have all been cannulations of the main pancreatic duct with polyvinyl plastic tubing after avulsion of the accessory ducts; the majority flowing two to four weeks, one lasting eight weeks. Three duodenal explants (an exteriorized button of duodenum containing the main pan-

creatic duct) failed. The difficulty of maintenance of pancreatic fistula dogs seems to have been best handled by returning the pancreatic juice through a polyvinyl gastric fistula to which the pancreatic cannula is connected constantly between experiments. Pancreatic juice is collected on ice and enzyme activity is preserved by adding an equal volume of glycerin. Volumes are greatest after meals, secretin, histamine and alcohol. Enzymes (amylase, lipase and trypsin) are highest after pilocarpine, olive oil, sham feeding and insulin hypoglycemia. The three enzymes tend to run parallel, however, for example, a fat meal may elevate lipase more than amylase and trypsin. Specific gravity and total protein determinations have been unrevealing.

*Sweat gland activity of dogs before and after cinchophen-induced ulcers.* A. H. RYAN AND L. M. WIDROW (by invitation). Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

The sweat glands of the dog have had very little study. Electrical resistance of the skin is a sensitive method of measuring changes in sweat-gland activity. It seemed that a study of the effect of cinchophen on foot pad resistance might shed light on its action on the sympathetic nervous system or on this type of gland. Resistance was measured daily by placing the feet in a circuit with 2 dry cells, a variable resistor and a microammeter. Cinchophen was given orally, 2 grams daily, 6 days per week, and measurements were generally made 18 to 22 hours after the preceding dose. Ulcers were produced in 9 normal dogs, and 4 with lumbar preganglionic sympathectomy. The hind-foot pad resistance was significantly decreased by cinchophen in each group, a response indicating increased sweat gland activity. Resistances lower than on any control day occurred within the first 4 days in 85% of the dogs. The following table shows the effects of both sympathectomy and cinchophen on pad resistance.

DOG	BEFORE SYMPATHECTOMY	AFTER SYMPATHECTOMY			DAYS OF SURVIVAL
		Mean—week before cinch.	Mean—week after cinch.	Change in percentage	
Ba.....	5575	75750	5280	-93.0	45
Fl.....	2800	65630	14875	-77.3	21
An.....	150000	1256660	38975	-96.9	27
Sp.....	10800	532500	64600	-87.9	8
Mean....	42293	482635	30933	-88.8	

The fore legs showed, in general, similar changes, but the results were not so clear cut. Cinchophen, therefore, has a demonstrable action on the sweat glands

which, on the basis of the present evidence, may be direct or indirect or both.

*Effect of para-aminobenzoic acid on the metabolism and excretion of salicylate.* ROBERT M. SALASSA AND JESSE L. BOLLMAN. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Following a report in 1946 that the oral administration of para-aminobenzoic acid increased the plasma salicylate-levels in man, an attempt was made to determine the mechanism by which para-aminobenzoic acid produced this effect. The salicyl fractions excreted in the urine and the plasma salicylate levels were determined in man and in the dog following the ingestion of sodium salicylate with and without para-aminobenzoic acid. In the dog the administration of para-aminobenzoic acid did not elevate the plasma salicylate value and did not alter the excretion of salicylate in the urine. In man the administration of para-aminobenzoic acid appeared to interfere with the conjugation of glycine with salicylic acid and resulted in a marked decrease in the quantity of salicyluric acid appearing in the urine. Kapp and Coburn have shown that man normally excretes about 80% of ingested salicylate in a form that contains an intact salicyl radicle and that approximately 50% of this is salicyluric acid, 25% salicyl glucuronate and 25% free salicylate. Para-aminobenzoic acid administration did not alter the excretion of the free salicylate or salicyl glucuronate fractions unless the pH of the urine was changed. When the urine was made strongly alkaline by giving large amounts of sodium bicarbonate with the para-aminobenzoic acid, the excretion of the free salicylate fraction increased enough to completely mask the effect of the decrease in excreted salicyluric acid on the renal clearance of total salicylate. The administration of para-aminobenzoic acid also appeared to interfere with the conjugation of benzoic acid with glycine as indicated by a decrease in the quantity of hippuric acid excreted in the urine in four hours following the ingestion of 6 gm. of sodium benzoate. These effects of para-aminobenzoic acid are temporary and reversible.

*Mechanism of apnea induced by electrophrenic respiration.* STANLEY J. SARNOFF. Harvard School of Public Health, Boston, Mass.

A new type of artificial respiration has been developed which depends upon the surgical insertion of a single electrode around one or both phrenic nerves and the application of an electrical current to the nerve. By means of a rotating potentiometer the voltage is made to vary between 0 and about 3 volts in such a way as to cause the diaphragm to contract and relax in a smooth manner. The revolution rate of the rotating potenti-



ometer determines the rate of respiration and is readily adjusted. Since the tidal volume is (within satisfactorily wide limits) in direct relation to the peak voltage applied, the depth of respiration and minute volume are likewise easily controlled. The technique is capable of maintaining normal blood gas tensions in the absence of spontaneous respiration in the cat, dog, rabbit and monkey. An interesting by-product of the development of the technique was the observation that spontaneous respiration ceased immediately after the onset of electrophrenic respiration. This suppression of spontaneous respiration is reflex in nature since after vagotomy it no longer occurs.

*Thermal conductance of the colonic wall; magnitude of changes during peristalsis, graded hemorrhage and re-infusion.* H. SCARBOROUGH, M. ELKIN, H. A. BLISS, H. W. PARK (by invitation), AND E. M. LANDIS. Dept. of Physiology, Harvard Medical School, Boston, Mass.

Thermal conductance in the colon of anesthetized dogs was measured during hemorrhage re-infusion and death to determine whether heat transfer was sufficiently rapid and consistent to serve as a continuous measurement of intestinal (splanchnic) blood flow in unoperated animals and in man. The thermal flowmeter consists of a hollow water-tight lucite chassis, 10 x 1.7 cm., carrying a wire coil which provides a constant known output of heat distributed throughout 34 ml. of water with which the thin rubber balloon enclosing the whole instrument is inflated. The water is mixed by a pulsating pneumatic device. Mounted near the middle of the chassis is one end of a 6-10 unit thermopile which with the heater, is protected from contact with the balloon by a metal guard. The other junctions of the thermopile are mounted on the surface of a solid lucite tip 5 x 1.2 cm., so as to be in contact with the gut wall, and hence at local body temperature when the tip is inserted 25 to 30 cm. up the colon. From the output of heat (e.g. 0.288 cal/sec.) between the temperature of the tip and the water in the balloon (e.g. 1.644°C.) as recorded continuously by an ink-writing potentiometer, the overall thermal conductance,  $C = \text{cals/sec.}$  In rapidly flowing water the  $C$  for such instruments was 0.600 to 0.700 cal/sec/°C.Δt. The overall  $C$  was 0.150 to 0.250 in the colon of living dogs and less than 0.100 in the colon of dead dogs. When heat was removed from the balloon mainly by conduction (wrapped in cotton-wool in water bath)  $C$  lay between 0.070 and 0.090, and by convection (bare in still water) 0.240 to 0.270. Peristalsis, always recorded throughout, usually increased  $C$ , whether due to movement artefact or active hyperaemia cannot at present be said. Atropinization

produced relatively constant  $C$  for long periods. Graded hemorrhage in normal or atropinized dogs decreased  $C$  often in stepwise fashion; re-infusion increased  $C$  rapidly and conspicuously.

*Patterns of cutaneous hyperalgesic points obtained by stroking the trunk with a sharp stylus.* ALFRED A. SCHILLER. Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.

Light linear centripetal stroking with a sharp stylus of the anterior and posterior surfaces of the trunk in an approximately horizontal plane may elicit a *point* of hypersensitivity along the path of the stroke. When the trunk is systematically stroked, the hypersensitive points tend to bilaterally parallel the midline, lying 2-4 cm. laterally. Over the back another array of points angle bilaterally from the upper thoracic spinous processes to the roots of the scapulae, and then run obliquely caudad to join the paramidline points in the region of the lumbar vertebrae. The only constant and appreciable deviation from the bilaterally symmetrical representation of hypersensitive points occurs in the region of the precordium where they are elicited farther to the left, frequently outlining the left border of the underlying heart as determined roentgenographically. With the exception of the precordial deviation the pattern of sensitive points obtained by stroking closely parallels the anatomical distribution of the sites of superficial emergence of the cutaneous branches of the somatic spinal nerves. The painful quality of the sensation and the latency in perception implicates the pain receptors as the principal mediators of the stroking hyperalgesia phenomenon. It is postulated that point hyperalgesia results when adjacent cutaneous receptors are rapidly and consecutively stimulated (stroking), permitting delicate sensory discrimination of densely populated receptor areas from sparsely innervated regions. In the precordium visceral (cardiac) afferent activity may raise the CES and lower the stimulus threshold in the same cutaneous segmental districts to account for the lateral deviation described.

*Analysis of the nerve membrane current-voltage characteristic as a non-linear 'impedance'.* OTTO H. SCHMITT. Dept. of Physics, University of Minnesota, Minneapolis.

It has been shown that much detailed information concerning the electrical properties of the excitable nerve membrane can be deduced by careful study of the exact shape of the action potential and its first two derivatives. Values for membrane current, charge, resting potential, and electric energy release during excitation are among the quantities which may be evaluated. By identifying the membrane static



capacitive charging current with the first derivative potential form it is possible to isolate the active non-linear impedance portion of the membrane excitation cycle and thus approach closer to a direct measurement of the energy yielding process in the membrane.

*Relation between local polarization and spike height in single medullated nerve fibers.* GORDON M. SCHOEPFLE AND JOSEPH ERLANGER. Washington Univ. School of Medicine, St. Louis, Mo.

A quantitative investigation of the relation between local polarization and spike height in single medullated nerve fibers was confined principally to the most irritable fiber in the phalangeal nerve of the green frog. Electrode alignment is indicated as follows: anode of the stimulating circuit, stimulating cathode, grounded lead and polarizing electrode in common, distal recording electrode and finally, the distal polarizing electrode, maintained at least 10 megohms above ground through the polarizing circuit. Anodal increments amounting to 80% of normal spike height and cathodal decrements of 60% were obtained with currents just short of blocking intensity. The relation between spike height  $h$  and polarizing voltage  $v$  conforms to the equation  $h = h_0 \pm kv$  in which  $h_0$  is the normal spike height and  $k$  is a constant depending only on direction of membrane current flow. The membrane resistance thus appears to be linear, but rectification is manifest in 24 of the 27 fibers investigated, the  $k$  factor being the greater for inwardly directed currents. The tangent ratio, or ratio between the two  $k$  values for each fiber was found to be unchanged on subjecting the nerve to 95% oxygen and 5% carbon dioxide or to Ringer's solution containing twice the usual amount of potassium, or to crushing under the distal polarizing electrode. Differences in extent of rectification are apparently not correlated with seasonal variations.

*Rôle of facilitatory reticulo- and vestibulo-spinal systems in maintaining spasticity.* L. H. SCHREINER (by invitation), D. B. LINDSLEY AND H. W. MAGOUN. Depts. of Anatomy and Psychology, Northwestern Univ., Medical School, Chicago, Ill.

The basic feature of the spastic state is an exaggeration of spinal stretch reflexes which are most pronounced and persistent in the antigravity muscles. Requisite to the appearance of spasticity is the elimination of central suppressor influences, release from which permits stretch reflexes to become exaggerated. Equivalently requisite is the maintained and now unopposed influence of central facilitatory systems, including the reticulo and vestibulo spinal systems, for their influence is the actual factor responsible for reflex exaggeration in spasticity. From this point of view, the therapeutic relief of spasticity in clinical conditions, might

find a logical point of attack in reducing the activity of the central facilitatory systems which are responsible for maintenance of the spastic state.

*Pyruvate metabolism in colpidium campylum.* GERALD R. SEAMAN. Biological Laboratory, Fordham Univ., and Marine Biological Laboratory, Woods Hole, Mass.

It has been shown that *Colpidium* is capable of synthesizing lipids from protein (*Biol. Bull.* 94: 29). Since pyruvate, through the tricarboxylic acid cycle, is the link between protein and carbohydrate metabolism, a study of this cycle was made in *Colpidium* as the first step toward the elucidation of the pathway of lipid synthesis in this organism. Pyruvate is rapidly metabolized; 12.5 millimoles are utilized in 4 hours by  $25 \times 10^4$  cells. Malonic acid inhibits the pyruvate effect (increased oxygen consumption and pyruvate utilization). The malonate effect is overpowered by the addition of fumarate. Succinate is formed upon incubating cells with fumarate, pyruvate, and malonate. The amount of  $\alpha$ -ketoglutarate formed from pyruvate is increased four-fold upon the addition of fumarate. When 10 millimoles of oxaloacetate are incubated with  $46 \times 10^4$  cells, in four hours there is recovered 0.3 millimoles of fumarate, 0.8 millimoles of malate, 3.6 millimoles of pyruvate and 1.7 millimoles of lactate. All the oxaloacetate added is utilized, none being recovered. The transamination system, pyruvate + glutamate = alanine +  $\alpha$ -ketoglutarate occurs in *Colpidium*. It has not been possible to recover acetate when cells are incubated with pyruvate and ammonium chloride.

*Gastric and esophageal secretion in the frog.* MARY SHELDON (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Heidenhain and other early investigators believed that in the frog the gastric body cells produce acid only, all pepsin coming from the esophageal glands. More recent investigators including Bensley and Friedman claim that both acid and pepsin are produced by the same gastric body cell. The object of this study was to confirm one of these views. In the stomach two kinds of mucous cells were found but no canalicular apparatus could be demonstrated in the body cells to show that they were typical parietal cells. Large heavily stained zymogen granules were found completely filling the esophageal gland cells. Small lightly stained granules were scattered in the gastric gland body cells in the fundic portion of the stomach. Microscopic examination of fresh gastric mucosa from a frog injected with neutral red showed acid secreting cells excreting the dye in some areas. Pepsin deter-

minations made from mucosal extracts and secretions showed high peptic activity in the esophagus and some in the stomach. Histamine increased the pepsin concentration of juice from the esophagus only, but caused some increase in volume of gastric secretion and a large rise in total acidity. No decrease in pepsin was found in the gastric juice of frogs after continuous histamine induced secretion for three days which indicates that the stomach does secrete pepsin. Thus these experiments support the view that the one type of cell in the body of the frog gastric glands secretes both acid and pepsin.

*Experimental miliaria in man: II. Production of sweat retention anidrosis and vesicles by various kinds of injury.* WALTER B. SHELLEY, PETER N. HORVATH (by invitation), FRED D. WEIDMAN (by invitation), DONALD M. PILLSBURY (by invitation).

A study has been made of the effects of various agents on the human sweat-gland apparatus when applied locally to the skin. They are as follows: 1. iontophoresis; 2. ultraviolet light; 3. heat; 4. solid CO<sub>2</sub>; 5. maceration; 6. adhesive tape; 7. AlCl<sub>3</sub> 20%; 8. soap; 9. fat solvents. No immediate alteration was noted in the normal sweating processes when the subject was stimulated to vigorous sweating by means of heat. However, within three to five days definite changes were observed within the areas treated. They varied from slight to marked anidrosis, and in the areas of greatest anidrosis, small clear, superficial vesicles developed. The vesicles did not appear in areas in which the secretion of sweat was locally inhibited by atropine. These phenomena could be demonstrated repeatedly over a period of one to two weeks, after which the sweat-function returned to normal. Microscopic study revealed tiny plaques of para and hyperkeratotic stratum corneum in the treated areas. The vesicles were situated entirely within the stratum corneum and serial sections demonstrated that they were directly connected with the sweat ducts. It would appear that the factor common to all stimuli applied is that of minor irritation, and the plugging of the sweat-gland apparatus is part of the response of the skin to this irritation. Evidently various kinds of stimuli can provoke the phenomenon of sweat-retention anidrosis.

*A new method to measure elastic properties of skeletal muscle in situ.* ERNST SIMONSON, JOSEPH BROZEK AND ANCEL KEYS. Laboratory of Physiological Hygiene, Univ. of Minnesota, Minneapolis.

The method is based on the measurement of the deformation of a muscle *in situ*, produced by a falling hammer striking the surface of the muscle. Evidence is presented that the measurement of the total contact

time between hammer and muscle, and of the rebound give a fairly good approximation of the actual deformation curve. The contact time between hammer and a metal plate in contact with the muscle is measured electrically. During the contact, a condenser is charged from a battery and later discharged into a ballistic galvanometer so calibrated that the contact time can be read from the scale in  $\sigma$ . The rebound is measured mechanically on a scale as a quotient of fall height: rebound height. The effect of hammer weight, fall height, size and shape of the contact plate was studied and the optimum conditions for the standardization of the method are demonstrated in two models, one for measurement of biceps muscle elasticity in sitting position, and the other one for measurement of leg muscle elasticity in lying position. Provisions are made for variation of muscle length and tension. The method is simple, the readings can be made within a few seconds. The size of the 'chance' variations is small compared to the changes produced by alterations in muscle tension. The method is sensitive enough to record small variations of muscle tension, and the time of contact is too short for interference of voluntary or reflex alterations of muscle tension.

*Observations on the curare-like action of thiamine.*

JAY A. SMITH (by invitation), PIERO P. FOA AND HARRIET R. WEINSTEIN (by invitation). Chicago Medical School, Chicago, Ill.

When thiamine is injected intravenously in large doses (50 mg/kg. or more) bradycardia, hypotension, vasodilation, and inhibition of respiration are produced. These effects are transitory if artificial respiration is provided. Recent experiments have shown that thiamine exerts neuromuscular block typical of curare, and that, conversely, the symptoms of thiamine toxicity can be produced by curare preparations, namely into costrin (Squibb) and D-tubocurarine (Squibb). Thiamine, 150 mg/kg., is approximately equivalent to D-tubocurarine, 0.15 mg/kg. Except for the great difference in dose, the results are similar; hypotension may last longer with thiamine, whereas the neuromuscular block may last longer with D-tubocurarine. In experiments with frogs, it was found that thiamine exerted typical curare effects. Intocostrin was found to be about 200 times as potent as thiamine on weight basis. In experiments on unanesthetized dogs, both thiamine and D-tubocurarine produce identical symptoms, namely, ataxia, inability to stand, staggering movements and exaggerated reflexes; D-tubocurarine is about 1000 times as potent as thiamine under these conditions. In other experiments the thiazole moiety (4-methyl-5-beta-hydroxyethyl thiazole, Merck and Company) was found to have curare-like action similar

to thiamine. It was only about one fifth as potent as thiamine in this respect. We conclude that when thiamine is given by rapid intravenous injection in large doses, its toxic effect is predominantly due to its curare-like action.

*Intravenous glucose tolerance tests in aged males.*

LUTHER E. SMITH AND NATHAN W. SHOCK. Institute of Experimental Biology and Medicine, National Institutes of Health, Bethesda, Md., and Baltimore City Hospitals, Baltimore, Md.

Intravenous glucose tolerance tests were done on 68 males in various age groups. There were 14 in the 20-29 year group, 12 in the 50-59 year group, 13 in the 60-69 year group, 17 in the 70-79 year group, and 12 in the 80-89 year group. All subjects were selected with history and physical examination. No subject was used who had a history of diabetes, jaundice, or glycosuria or who manifested edema, jaundice, any type of liver disease or glycosuria. A fasting femoral arterial blood sample was drawn, and 50 cc. of a 50% glucose solution in distilled water was injected into the right or left basilic vein. Arterial blood samples were drawn at 5 and 10 minutes after the start of the injection and every 10 minutes thereafter for 2 hours with a final specimen drawn at 2½ hours. The blood was heparinized and stored in ice, and at the end of the experiment true blood sugar was determined by Nelson's modification of the Somogyi procedure. The mean fasting value varied between 79-82 mg.% for all the groups with no significant difference between any group. There was a progressive increase in the mean time in minutes to return to the fasting level for each group with a significant difference at the 1% level between the 20- to 29-year group and the 70- to 79-year group, and between the youngest group and the 80- to 89-year age group.

*On the maintenance of spontaneous activity within the cerebellum.* RAY S. SNIDER AND EARL ELDERED (by invitation). Northwestern Univ. School of Medicine, Chicago, Ill.

Preliminary studies on the mechanisms involved in the maintenance of the fast spontaneous electrical activity of the cerebellum have been made upon cats, either decerebrated or under various depths of barbiturate anesthesia. The electrical activity was amplified thru Grass Model III amplifiers and was observed directly on a three-channel cathode ray oscilloscope. That the activity is not dependent upon driving mechanisms outside the cerebellum is shown by the fact that it continues after bilateral destruction of all cerebellar peduncles. That it is not dependent upon a cerebellar-cortex to cerebellar nucleus back to cortex circuit is shown by the fact that it continues following

destruction of all cerebellar nuclei. It is concluded that all mechanisms necessary for the maintenance of this activity are located within the cerebellar cortex, and evidence is presented to show that the responsible units are either the granule cell plus glomerulus and/or the Purkinje cell.

*Blood and tissue lipids in the chick fed cholesterol in various forms.* J. STAMLER, C. BOLINE, E. LEVINSON, M. DUDLEY AND L. N. KATZ. Cardiovascular Dept., Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

Five groups of 5 Leghorn cockerels each, 5-8 weeks of age, were fed *ad libitum* for 10 weeks on a commercial chick starter mash supplemented with 2% cholesterol in various forms, including: a) amorphous cholesterol; b) crystalline cholesterol; c) cholesterol dissolved in ether and re-evaporated after admixture with the mash; d) crystalline cholesterol with cottonseed oil. In addition a control series of 5 birds with no supplement was run. Food intake and bird weights were recorded periodically. Plasma cholesterol (total and free), phospholipid and total fatty acids were measured at regular intervals. Liver, aorta, heart, kidney, intestine and carcass were analyzed at the end of 10 weeks for total and free cholesterol. 1) Groups a, b and c (cholesterol without oil) responded similarly, developing a progressive moderate hypercholesterolemia and hyperlipemia. Group d (cholesterol with oil) developed a far more marked and rapidly progressing hypercholesterolemia and hyperlipemia. In all groups the plasma free cholesterol ratio remained unchanged. The percentage increase in plasma cholesterol in all cholesterol-fed groups exceeded significantly the percentage rise in phospholipid and neutral fat. 2) Early atherosclerosis of the aorta and other great vessels was noted grossly in all cholesterol-fed groups, its extent and severity being significantly greater in group d (cholesterol with oil). 3) Tissue cholesterol analyses reveals an organ lipoidosis in all cholesterol-fed groups, which can be generally related to the blood cholesterol levels. The increase in lipid in the various tissues was not uniform, being much greater in the aorta and liver. Accumulation of esterified cholesterol was the characteristic feature of the organ lipoidosis. The occurrence of atheroma would appear to be related to: 1) the hypercholesterolemia; 2) the apparent disturbance in the ratio of plasma lipid fractions; 3) the organ lipoidosis, particularly of the liver and aorta; 4) the apparent disturbance of the normal tissue cholesterol esterification ratio.

*Pressure and activity recordings along the gastrointestinal tract in man.* F. R. STEGGERDA AND W. C. CLARK (by invitation). University of Illinois, Urbana.

It was previously reported that satisfactory pressure and activity recordings of the lower colon in man could be made with a water manometer connected to an open-tipped tube inserted five inches beyond the anal sphincter. The method was satisfactory except that plugging of the tube occurred when the colon was not completely empty, or the fecal material thin and watery. With this objection it is obvious that the technique could not be used in recording pressures in other places along the gastrointestinal tract because of the fluidity of its contents. Recently a water capsule (1 cm. long and .5 cm. in diameter) attached to the end of a rubber catheter connected to an electronic recording pick-up was devised which records pressure and activity along the gastrointestinal tract continuously without any interruptions. A comparison between the open-tipped water manometer and the closed water capsule electronic recording method showed that pressures in the colon of man with the latter method is definitely higher than those recorded by the water manometer. Recording of stomach pressure and activity as well as the changes that occur while the water capsule is passing through the pyloric sphincter region and entering the small intestine were made. Changes in position while lying horizontally on a cot do not significantly alter pressures in the gastrointestinal tract.

*Effect of increased carbon dioxide in inspired air on gastric emptying in dogs.* J. CLIFFORD STICKNEY, DAVID W. NORTHUP AND EDWARD J. VAN LIERE. Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown.

The normal gastric emptying time of 4 dogs was determined fluoroscopically after intubation by stomach tube of 50 ml. of a suspension of BaSO<sub>4</sub> in 20% gum acacia solution. Immediately after intubation, the dogs were kept in a well ventilated chamber in which they were at other times exposed to increased concentrations of CO<sub>2</sub> in the inspired air. The effect of CO<sub>2</sub> was determined by placing the dogs after intubation into a chamber through which were led mixtures of CO<sub>2</sub> in O<sub>2</sub> of the desired concentrations. Twenty-five determinations of gastric emptying were made while the dogs were exposed to concentrations of CO<sub>2</sub> in the inspired air ranging from 6.6-12.3%. Twenty control observations were made on the same dogs. The responses to CO<sub>2</sub> were rather variable, not only in the individual dog but in the group as well. When the CO<sub>2</sub> range was 6-8%, the average increase in gastric emptying time varied from 15 to over 100% in the 4 dogs; between 8 and 10% CO<sub>2</sub> the increase was 67 to over 288%; between 10 and 12% CO<sub>2</sub> the increase was 73 to over 300%. The delay in gastric emptying

appeared to be proportional to the concentration of CO<sub>2</sub> in the inspired air over the range studied.

*Influence of estrogens on x-ray toxicity.* R. L. STRAUBE (by invitation), H. M. PATT AND M. N. SWIFT (by invitation). Argonne National Laboratory, Chicago, Ill.

It has been reported that a single intramuscular injection of alpha estradiol-benzoate nine days prior to irradiation decreased radiotoxicity in male Swiss mice, whereas estrogen administered on the day of irradiation potentiated sensitivity (*Endocrinology* 32: 161, 1943). We have extended these findings and initiated investigations designed to elucidate the mechanism of this estrogen effect. We have observed a similar protective estrogenic effect in both male and female mice of the CF<sub>1</sub> strain with either alpha estradiol-benzoate or the synthetic estrogen Benzestrol (Schiefelin), 0.1 mg. alpha estradiol-benzoate or 1.0 mg. of Benzestrol, 10 days prior to x-irradiation (500 r) giving essentially complete protection. When the estrogen was given five days before irradiation a probably significant but somewhat lesser effect was evidenced. Administration at the time of irradiation gave equivocal results. Since the estrogen effect may be non-specific, other steroids are being investigated. Preliminary experiments indicate that progesterone (0.1-0.2 mg/25-gm. mouse) has little if any protective action. Pilot studies indicate that a single dose of estrogen alone (0.1 mg. I.M.) has no appreciable effect on the weight of spleen, inguinal nodes, or kidneys, but does increase adrenal weight and accelerate thymic involution. An early transient leukocytosis followed by a severe leukopenia reaching its apogee ten days post injection with a recovery toward normal at fourteen days, has been observed in such estrogen-treated animals.

*Aortic deposition of cholesterol in experimental atherosclerosis.* A. N. TAYLOR AND JOE A. STEWART (by invitation), Dept. of Physiology, University of Oklahoma School of Medicine, Oklahoma City.

As a part of a study of the flow pattern in major blood vessels, the cholesterol content of four selected regions of the aortae of normal and cholesterolized rabbits was determined. Thirteen rabbits received 0.6 gm. of cholesterol in vegetable oil added to a daily base diet of 75 gm. commercial rabbit chow; 10 rabbits received an equal amount of vegetable oil added to the base diet; and 13 rabbits received only the base diet. Serum levels of total cholesterol, which were determined periodically, averaged at the end of the test period of 100 days, 2000 mg.% for the cholesterolized animals as compared with 60 mg.% for the two control groups. The animals were sacrificed, each

aorta removed and divided into four regions; each region was dried to constant weight, subjected to alkaline digestion, and its cholesterol content determined by Sperry's modification of the Lieberman-Burchard reaction. Cholesterol content of the aortic regions of the vegetable oil-base diet group did not differ significantly from that of the base diet group. Average cholesterol content expressed in milligrams % of the dry weight of each of the four regions was as follows (values for the cholesterinized rabbits are italicized): 1) ascending aorta and arch, *3.47* and *0.17*; 2) thoracic aorta below the arch, *1.29* and *0.17*; 3) upper abdominal including the renal arteries, *1.50* and *0.23*; 4) lower abdominal aorta and proximal iliac arteries, *1.11* and *0.30*. A relationship between cholesterol deposition and areas of probable turbulent blood flow is indicated.

*Studies of the pulmonary and systemic arterial pressure in cases of patent ductus arteriosus with special reference to effects of surgical ligation.* B. E. TAYLOR (by invitation), A. A. POLLACK (by invitation), H. B. BURCHELL, O. T. CLAGETT (by invitation) AND E. H. WOOD. Mayo Foundation, Rochester, Minn.

A study has been made of the blood pressure in the pulmonary artery and systemic circulation in 11 cases of patent ductus arteriosus during surgical ligation of the ductus. Preoperative cardiac catheterization studies were carried out in 4 instances. Pulmonary artery pressures were measured in the open thorax by means of an intra-arterial needle and a strain-gage manometer before and after closure of the ductus. Systemic blood pressures were recorded during the operation by means of an indwelling needle in the radial artery. These pressures and the heart rate, electrocardiogram and respiration all were recorded on the same photographic paper. In 4 patients in whom cardiac catheterization studies were carried out preoperatively, the average flow through the patent ductus arteriosus was calculated to be 7.5 (3 to 17.7) l/min. In 6 patients calculations based on determinations of oxygen saturation of systemic arterial, pulmonary arterial and mixed venous blood indicated that the average flow through the ductus arteriosus was 49 (29 to 75) % of the left ventricular output. The average outside diameter of the ductus arteriosus measured at operation in these cases was 1.1 (0.8 to 1.3) cm., as compared to the average value of 1.0 (0.75 to 1.3) cm. for the complete series. The average mean systemic to pulmonary artery pressure gradient was 60 (16 to 82) mm. of mercury. In these cases there was a direct correlation (correlation coefficient:  $0.9 \pm 0.25$ ) between the ductus flow in cc/100 cc. of left

ventricular output) and the square of the radius of the ductus multiplied by the square root of the pressure gradient. Closure of the ductus arteriosus produced an immediate increase in the systemic blood pressure and a decrease in the pulmonary arterial pressure in every instance. These changes could be repeated at will by opening or closing the duct with a rubber-covered surgical forceps. In this series of patients the average immediate increase in systemic pressure coincident with closure of the ductus was  $8.9 \pm 1.9$  (3.2 to 20.7) mm. Hg systolic, and  $11.9 \pm 2.7$  (3.1 to 30.5) mm. Hg diastolic. The average immediate decrease in pulmonary artery pressure was  $4.3 \pm 1.2$  (-0.5 to 10) mm. Hg systolic, and  $5.8 \pm 1.3$  (1.1 to 10) mm. Hg, diastolic.

*Physiological properties of regenerating nerve fibers.*

J. D. THOMSON (by invitation) J. A. MORGAN (by invitation) AND H. M. HINES. Dept. of Physiology, State Univ. of Iowa, Iowa City.

Tibial nerves of rats were crushed 1, 2 or 3 times at the same point at 42-day intervals, and gastrocnemius weights and isometric tensions were measured at 21, 42 and 84 days after the final lesion. Values for muscle weight and tension, recorded as percentage of contralateral control, showed that the gastrocnemius can undergo at least 3 consecutive denervations and recoveries without impairment of its regenerative capacity. Twitch tension values in % of tetanus tension (tetanizing frequency 120/sec.) suggest that muscle's ability to summate twitches is not impaired during regeneration, since values at 14-17, 20-22 and 25-28 days are of nearly the same magnitude as control values. The slightly higher values at 20-22 and 25-28 days may mean that optimal tetanizing frequencies for regenerating nerve and muscle are lower than 120/sec., and that if the optimal frequency were used the values might be lower. The rate of transmission fatigue (stimulus frequency 120/sec., ether anesthesia) was much more rapid 21 days after a crush lesion than in normal controls; it was less rapid at 28 days than at 21 days. When intraperitoneal Dial was used as anesthetic, transmission fatigue in normal rats occurred more slowly than under ether; at 21 days of regeneration the rate was more rapid than in controls for the first 5 seconds, then paralleled the control curve from the 5th to 10th seconds.

*A comparison of action of anticonvulsants on the excitability of peripheral nerve and of motor cortex.* JAMES E. P. TOMAN AND CHARLES D. HENDLEY. Dept. of Physiology, Univ. of Utah School of Medicine, Salt Lake City.

The following observations suggest that anticonvulsants may prevent neuronal alterations produced

by excessive electrical or chemical stimulation without impairment of normal function: *a*) Ringer's solution saturated with Dilantin (diphenylhydantoin) was without notable effect upon threshold, spike amplitude, spike duration, recovery, and conduction velocity of frog sciatic nerve. However, concentrations as low as 0.04 mM/l abolished repetitive responses and extraordinary supernormality produced by brief high voltage shocks. Dilantin also prevented hyperexcitability and repetitive firing in nerves treated with isotonic sodium phosphate solution. These effects occurred with low concentrations of many other anticonvulsants including Phenurone, phenobarbital, Mebaral, Mesantoin, and Epidon. *b*) Dilantin did not affect the exchange of radioactive sodium in frog nerve in Ringer's solution. However, it prevented the increased rate of sodium exchange in phosphate solution. *c*) The frequency-threshold curve for non-convulsive localized movement produced by repetitive brief shocks to the cerebral cortex of the unanesthetized rabbit reaches a minimum at 100 c.p.s. After Dilantin treatment the threshold in the minimum region was not raised significantly, but there were significant increases at the extremes (15% at 1000 c.p.s.; 20% at 3 c.p.s.). *d*) The curve of recovery of responsiveness of the rabbit motor cortex following each stimulus in a repetitive series shows a period of supernormal excitability, with a peak at 2 to 4 msec. Dilantin was without effect on this process. However, Dilantin has been previously shown to prevent high frequency seizure discharges in the rabbit EEG.

*Effect of hypophysectomy on the electromyogram.* CLARA TORDA AND HAROLD G. WOLFF. New York Hospital and the Depts. of Medicine (Neurology) and Psychiatry, Cornell Univ. Medical College, New York City.

Some aspects of myasthenia gravis (e.g. occasional increase of thymus, increase of lymphoid tissue, decrease of acetylcholine synthesis) suggest that the decreased ability of patients with this muscle disorder to maintain an adequate muscle function during prolonged work is a result of a partial dysfunction of the pituitary gland. One of the objective tests characteristic of patients with myasthenia gravis is a decline of the amplitude of muscle action potential during repetitive indirect stimulation. In the following it was ascertained whether removal of the pituitary gland induces a similar dysfunction of the muscle. Therefore, electromyograms of 33 hypophysectomized rats were compared with electromyograms of 20 control rats operated on with the same technic except for the removal of the pituitary gland. Electromyograms were recorded from the gastrocnemius muscle during

a few minutes' stimulation of the sciatic nerve with a current having a repetition rate of from 3 to 30 pulses per second and being of 'supramaximal' intensity. The area and amplitude of the muscle action potential of the control rats remained either unaltered or decreased somewhat, e.g. the decrease averaged 19% at the end of a 3-minute stimulation period with 11 pulses per second. The amplitude and area of the muscle action potential of the hypophysectomized rats decreased with low frequency stimulation and the decline of the electromyogram increased with the frequency of stimulation. The decrease averaged 82% at the end of a 3-minute stimulation period with 11 pulses per sec. Therefore, a dysfunction of the pituitary gland may be linked with a muscle dysfunction. This effect is probably not exerted through the adrenal gland alone.

*X irradiation of the hypophysectomized rat.* E. B. TYREE (by invitation), M. N. SWIFT (by invitation) AND H. M. PATT. Argonne National Laboratory, Chicago, Ill.

We reported previously that x-radiation in common with other types of stress appears to result in an increased demand for the adrenal cortical hormone (*Am. J. Physiol.* 150: 480, 1947). It seemed of interest, therefore, to determine whether hypophysectomy would prevent the adrenal response to x-radiation and to note whether survival and some of the typical changes in organ weights would be altered under these conditions. Sixty white male rats (200-300 gm). received 750 r total-body x-irradiation, 30 having been hypophysectomized one week prior to exposure. Animals were sacrificed at 3 hours and 4 days after irradiation and comparison was made with appropriate groups of nonirradiated rats. Hypophysectomy prevented adrenal changes (decreased adrenal cholesterol concentration and increased adrenal weight) seen in intact rats after x-irradiation, but did not alter the degree nor time course of the splenic and thymic involution. X-ray toxicity appeared to be potentiated by pituitary ablation. Forty-five % of 20 hypophysectomized irradiated rats died 3 to 4 days after the exposure whereas none of the intact irradiated animals succumbed until 6 days after irradiation and there was only a 30% mortality at 16 days. It remains to be determined whether the adrenals of the 7-day hypophysectomized rat do not respond to x-radiation because they are generally less sensitive owing to removal of pituitary influence or because their stimulation after irradiation is mediated solely by the adrenotrophic hormone.

*Effect of explosive decompression on the temperature of the lungs.* EDWIN G. VAIL (by invitation) AND

FRED A. HITCHCOCK. Dept. of Physiology, Ohio State Univ., Columbus.

Experiments on explosive decompression conducted in this laboratory, made it desirable to investigate lung temperatures. All experiments were conducted on anesthetized dogs. Lung temperatures were measured with thermistors. A thermistor located in the trachea recorded the temperature of inspired and expired air. The temperature of expired air never reached the temperature recorded in the deep lung. Measurements of temperature at various levels in the pulmonary tract gave a mean temperature in the trachea of  $34.5^{\circ}\text{C}$ , and in the deep lung of  $37.1^{\circ}\text{C}$ . At explosive decompression, tank temperatures were found to decrease an average of  $2.0^{\circ}\text{C}$  for all terminal pressures. The greatest drop occurred in 0.2 seconds. Respiratory patterns showed many variations. The point in a respiratory cycle at which the explosion occurred had a pronounced effect on the respiratory pattern. These effects may be due to an exaggerated stimulation of the Hering-Breuer reflex. The average temperature in the deep lung before explosive decompression was  $39.0^{\circ}\text{C}$ , and the average decrease for pressures lower than 220 mm. Hg was  $0.5^{\circ}\text{C}$ . In all experiments following explosion to 30 mm. Hg the temperature in the trachea remained at a low level until recompression. The average decrease in tracheal temperature was  $8.8^{\circ}\text{C}$ . Although attempted respiratory movements were observed in these animals no ventilation of the lungs occurred. The temperature changes in the lungs occurred in two phases. The first phase probably is due to the expanding lung gases, and the second to the vaporization of water.

*Duration of anti-secretory action of enterogastrone and urogastrone in rats and a comparison of potency in rats and dogs.* F. E. VISSCHER AND M. I. GROSSMAN. The Upjohn Company, Kalamazoo, Mich., and Dept. of Clinical Science, Medical School, Univ. of Illinois, Chicago.

The duration of anti-secretory action of preparations of enterogastrone and urogastrone has been observed in pyloric ligation rats. After a 40-hour fast, adult male rats under ether anesthesia were injected intravenously. Groups of 6 to 8 rats were taken for pyloric ligation under ether anesthesia at different intervals after injection (zero hour, two hour, and four hour). The inhibitory ability of two samples of enterogastrone was greatest during the first two hours after injection; for two samples of urogastrone this action was greatest during the second two-hour period after injection. There was evidence of activity of both urogastrone and enterogastrone during the third two-hour period. The anti-secretory activity of six samples of entero-

gastrone has been compared in the rat and dog. A rat unit is the amount in mg. to inhibit by 50% the volume of gastric juice secreted by the pyloric ligation rat; secretion is measured during the first two hours after injection, and compared with uninjected or gelatin injected controls. Values for 50% inhibition are found by interpolation or extrapolation of figures for inhibition (determined in six or more rats at two or more dosage levels). In general, poor correlation was obtained between unitage in the dog and rat, although a dialysed enterogastrone preparation which was most active in the dog was also most active in the rat.

*Inferior caval and portal pressures in relation to the formation of ascites in the dog.* WADE VOLWILER, J. H. GRINDLAY, AND J. L. BOLLMAN. Division of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Pressures in the inferior vena cava and portal vein of the standing dog were measured with a water manometer attached to flexible polythene tubing previously inserted into these veins at laparotomy. Data were obtained in 5 normal dogs and in 30 animals with previous ligations, constrictions, or anastomoses of the main venous channels around the liver. Ascites, marked hepatic congestion, and engorged hepatic lymphatics were regularly produced by the progressive constriction of the thoracic inferior vena cava from the fibrous reaction to a cellophane band. Most, but not all, of these dogs developed a marked hypoproteinemia with normal albumin-globulin ratio. Inferior caval and portal vein pressure readings in such animals, though elevated, were found to be no higher than in other types of preparations wherein ascites did not occur. Anatomic studies were made of the venous collateral circulation. Attention is called to the anterior spinal veins as important collaterals to the obstructed inferior vena cava in the transfer of blood from the lower cava to the azygos vein. By none of the operations upon the venous circulation was it possible to produce a marked portal hypertension comparable to that which has been reported as measured at the time of laparotomy in some human patients with esophageal varices.

*Effects of intravenous histamine on the peripheral circulation in man.* K. G. WAKIM, G. A. PETERS, J. C. TERRIER AND BAYARD T. HORTON. Mayo Clinic and Mayo Foundation, Rochester, Minn.

The effects of continuous intravenous administration of histamine diphosphate on skin temperature, blood pressure, heart rate and blood flow were studied on patients who were receiving the drug for therapeutic purposes. The drug was administered to each patient



in a solution of 1:250,000 in saline at successive rates of 0.004, 0.008, 0.016 and 0.024 mg. of histamine/min., respectively. The duration of infusion at each rate was 20 minutes. Control values for skin temperatures, heart rate, blood pressure and blood flow were established before the infusion of histamine was started, and the observations were repeated at regular intervals thereafter for each of the periods of infusion at each of the four infusion rates and for 5 to 15 minutes after the infusion was stopped. The blood flow in all four extremities was determined by means of the venous occlusion plethysmograph with a compensating spirometer recorder. The cutaneous temperatures were recorded galvanometrically by means of skin thermocouples applied to the forehead, to the skin over the right and left deltoid muscles, and over the right and left quadriceps femoris muscles. Histamine produced cutaneous vasodilatation which appeared first over the face and neck of the patient and gradually extended downward over the upper extremities and thorax, reaching the lower extremities only toward the end when the higher rates of infusion were used. There was a definite increase in skin temperature and in heart rate, and a slight decrease in diastolic blood pressure. The blood flow in the four extremities gradually increased in proportion to the dosage used, until at the highest rate of infusion of 0.024 mg. histamine/min. the average increase in blood flow over the control values was 245% in the left arm, 120% in the right arm, 76% in the left leg, and 28% in the right leg, respectively. However, five minutes after the infusion of histamine was stopped, the blood flow averaged only +46% in each of the arms and +40% in the left leg, and +14% in the right leg. The changes in skin temperature, blood flow, heart rate and blood pressure gradually subsided, and the values returned toward the control level shortly after stopping the infusion of histamine.

*An analysis of the cardiovascular effects of the intravenous injection of small volumes of hypertonic solutions in the anesthetized dog.* WILLIAM W. WALCOTT AND INGRITH J. DEYRUP (by invitation). Dept. of Physiology, College of Physicians and Surgeons, and Dept. of Zoology, Barnard College, Columbia University, New York City.

The marked but transient fall in blood pressure following the intravenous injection of hypertonic solutions in mammals has been ascribed by different investigators to cardiac weakening or, alternatively, to reduction in peripheral resistance (*Am. J. Physiol.* 151: 516, 1947). We have noted that a characteristic diphasic fall in systemic arterial pressure follows the injection of 4 to 20 ml. of 5 to 20% NaCl or 50% glucose

in normal, sympathectomized or vagotomized dogs (nembutal anesthesia), confirming the conclusion of other workers that the hypotension is not reflex in origin. Heart rates varied slightly or were unchanged, whereas the femoral arterial pulse pressure was decreased in the first phase of hypotension, and significantly increased in the second phase. Throughout both phases, pulmonary arterial pressure was slightly elevated or unchanged, pulmonary arterial pulse pressure was increased, and both central and pulmonary venous pressures were somewhat elevated (membrane manometer records). These findings suggest that the first phase of fall in blood pressure may result from myocardial weakening, rather than from decreased venous return or vasodilatation. Vasodilatation must, however, play a part in the secondary fall in mean arterial pressure, when the femoral pulse pressure increases with unchanged or elevated heart rate. It has been noted that a severe, single phase, rather than diphasic, hypotensive response follows the intravenous injection of hypertonic solutions mixed with blood prior to injection. This phenomenon, which is being investigated further, may throw additional light on the mechanism of the characteristic fall in blood pressure after injection of hypertonic solutions.

*Effect of low temperature on the mechanical response and action potential of rat muscle.* SHEPPARD M. WALKER. Washington Univ. School of Medicine, St. Louis, Mo.

Male rats were anesthetized with 300 mg. of Na barbital/kg. and placed in a cold room until the rectal temperature decreased to about 22°C. The animals were removed from the cold room and the records were obtained from the gastrocnemius muscle by the time the rectal temperature had increased to approximately 26°C. The 'cooled' muscle showed a 50% increase of tension and about a 100% increase in contraction time and relaxation time over the normal muscle in response to single indirect stimuli. The developed tension resulting from 2 shocks with appropriate intervals was always more than double the developed tension of single responses in normal muscle. On the other hand, the developed tension induced by 2 stimuli with similar intervals was usually about 50% greater than the tension developed after single shocks in 'cooled' muscle. Tetanic stimulation at 125 stimuli/sec. for 0.2 sec. induced approximately equal development of tension in 'cooled' and in normal muscle though the rising phase and the falling phase of the tension curve was longer in 'cooled' muscle. The 'cooled' muscles showed only slight (less than 20%) increase of developed tension as a result of treppe in contrast to the 35 to 40%



increase seen in normal muscle similarly exercised. Exercise brought about a 300% increase of relaxation time and a slight decrease of contraction time of 'cooled' muscle. Similar exercise of normal muscle shortened both the contraction time and the relaxation time. Cooling under the condition of these experiments produced about 100% increase of the duration of action potentials obtained with belly and tendon leads.

*Effect of the lysozyme on the gastric mucosa.* K. J. WANG (by invitation) AND M. I. GROSSMAN. Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.

Acute experiments were carried out on fasting rats under nembutal. *Series I:* the rat's stomach was exposed for four to six hours to lysozyme, 2 mg/cc. in buffer pH 5.3. Out of six experiments, one rat showed ulcer, one erosion, and a third hemorrhage. In controls the stomach was exposed to buffer alone and no gross or microscopic changes occurred. *Series II:* the mucosa was exposed to lysozyme in buffer and the solution changed every half hour for three hours, followed by 0.2% pepsin in 0.15 N HCl for three hours, changed every half hour. All showed peptic ulcers. *Controls:* one group treated successively with buffer followed by acid-pepsin showed no ulcers. A second control group, treated successively with lysozyme in buffer followed by saline, showed slight erosion or hemorrhage. *Series III:* 0.15 N HCl and 0.2% pepsin and lysozyme together caused some damage in all cases, but definitely less than was caused by the two enzymes in separate solution. *Controls:* the same acid-pepsin without lysozyme, showed only slight change. Besides, the effect of lysozyme on dogs' gastric mucosa has been observed *in vitro* by incubating tissue sections prepared by the freezing-drying method in lysozyme. Most of the mucus in the surface epithelial cells showed vacuolization and the neck mucoid cells were poorly stained. *Controls:* sections incubated in buffer showed intensive stain of both the surface epithelial cells and the neck mucoid cells without vacuolization. It is concluded that the high concentration of lysozyme in buffer at pH 5.3 produced erosion and hemorrhage in the gastric mucosa, and increased the injurious action of acid-pepsin.

*Sensitivity of the esophagus to the acid-pepsin action.* OWEN H. WANGENSTEEN, HENRI SANCHEZ AND Y. SAKO. Dept. of Surgery, Univ. of Minnesota, Minneapolis.

The sensitivity of the esophagus to the action of gastric juice as compared to the sensitivity of the stomach and duodenum was studied. In rats, ligation of the duodenum close to the pylorus leads to ulceration of the esophagus and of the squamous portion of the

stomach. These results seem to be enhanced by the added action of histamine. When food protects the stomach of the animal against ulceration, the esophagus might become damaged in some animals. In dogs, ligation of the duodenum close to the pylorus, plus daily injections of histamine, leads to esophagitis and/or perforation of the esophagus when the stomach is still grossly normal. That the distention produced by the ligation is not in itself the cause of the lesions was evident when the ruptured strength of esophagus, stomach and duodenum was tested on normal animals and after ligation. Continuous drip of 0.1 N HCl solution or of gastric juice into the esophagus and stomach lead to ulceration and sometimes perforation of the esophagus while the stomach is still grossly normal.

*Motor responses of spatially transposed intestinal loops.*

D. H. WATKINS (by invitation) AND F. C. MANN. Institute of Experimental Medicine, Mayo Foundation, Rochester, Minn.

Exteriorized skin-covered loops of jejunum and ileum of dogs were studied in respect to their rate of rhythmic contractions and motor responses after feeding. The positions of the intestinal loops were then changed with respect to their distance from the pylorus. Loops which were originally high in the jejunum were transplanted without interference with their blood and nerve supply to a position low in the ileum, and vice versa. Although this operation of intercalary transplantation of intestinal loops carries several inherent risks, several preparations have remained in good condition four years after operation. The rate of rhythmic contractions in the transplanted loops was the same as in the original position minus one or two contractions/min. It is an inherent property of the loop and does not depend upon the relative position of the loop in the intestinal tract. The time of the motor response after feeding depends upon the relative position of the loop in relation to the remainder of the intestinal tract. The more oral the loop, the quicker does the motor response occur after feeding. These findings support the theory that following the ingestion of food, a wave of activity passes down the small intestine.

*Function of the pancreas of alloxan diabetic dogs studied by means of cross-circulation experiments.* HARRIET R. WEINSTEIN (by invitation), JAY A. SMITH (by invitation) AND PIERO P. FOA. Dept. of Physiology and Pharmacology, Chicago Medical School, Chicago.

In a preceding note cross-circulation studies on insulin secretion were reported. It was found that after an intravenous injection of 20% glucose (5 cc/kg.) into a normal dog (A) connected with a recipient dog (B) through a pancreatic-femoral anastomosis, the blood sugar of B decreases sharply. This was interpreted

as evidence of an increase in insulin secretion by the pancreas of *dog A*. When instead of normal animals, alloxan diabetic dogs are used as donors of pancreatic blood, the blood sugar of *dog B* increases instead of decreasing. If the alloxan diabetic donor and the recipient are connected through a mesenteric-femoral anastomosis the upper glycemia of *B* is much less pronounced and shorter lasting. The results indicate that the hyperglycemia observed in *dog B* is not due to the glucose injected into *A* and carried across the anastomosis, but is probably due to a hyperglycemic substance secreted by the alloxan resistant portion of the pancreas and similar to that found in most insulin preparations. The possibility that the pancreas might secrete a hyperglycemic substance is of great interest as it would explain why depancreatized dogs require less insulin than alloxan diabetic dogs and why a totally depancreatized man requires less insulin than patients with a moderately severe diabetes.

*Endocrine influences on renal function.* H. L. WHITE, PETER HEINBECKER AND DORIS ROLF (by invitation). Washington Univ. School of Medicine, St. Louis, Mo.

We have previously reported that the depression of renal function following hypophysectomy in the dog and manifested by striking falls in PAH, inulin and urea clearances and in PAH Tm and by a moderate rise in plasma NPN, with normal plasma Na and K levels, cannot be ascribed to thyroid or gonad regression due to loss of thyrotrophic or gonadotrophic hormones. The question of loss of adrenotrophic hormone or of some further anterior lobe principle acting directly on the kidneys or on tissues in general to produce increased renal activity was left unsettled. The present report presents evidence that the renal depression of hypophysectomy is not due to loss of ACTH. First, adrenal replacement therapy with DCA pellets adequate to maintain the observed renal functions of adrenalectomized dogs at or near normal has no protective effect against the depression of these renal functions following hypophysectomy. Second, stimulation of the adrenal cortex of hypophysectomized dogs by ACTH does not improve renal function as it should if the depression were due principally to adrenal deficiency. The inconstantly observed enhancing effects of ACTH on the renal functions of hypophysectomized dogs cannot be ascribed to adrenocortical stimulation, since such enhancing effects are also sometimes seen in adrenalectomized dogs. Furthermore, large doses of whole adrenal cortical extract supplementing DCA pellets do not improve the renal functions of hypophysectomized dogs. It is concluded that the loss of some further anterior lobe principle, not thyrotrophic, gonadotrophic or adrenotrophic, is principally responsible for the renal depression of hypophysectomy.

*Use of capacitance changes for the study of the mechanical activity and output of the heart.* WILLIAM V. WHITEHORN AND EDWARD PERL (by invitation). Dept. of Physiology, Univ. of Illinois, College of Medicine, Chicago.

The need of a technically simple method of estimating human cardiac output applicable to general clinical and laboratory use is evident. We have begun investigations of the use of capacitance changes in a condenser field containing the heart for this purpose. Using an improved method described in a companion paper, we have now recorded some 100 'cardiodielectrograms' (c.d.g.) on 30 essentially normal subjects. Recordings are technically easy, require a minimum of subject cooperation and yield a beat-to-beat record of cardiac activity. Outstanding difficulty with present apparatus is the necessity of suppressing respiration during recording. Simultaneous recording of phonocardiograms permits interpretation in terms of events of the cardiac cycle. Records reveal a typical general pattern resembling classical cardiometer curves but differing from these particularly in the early and late phases of systole probably because of movements of the heart and the presence of auricles and portions of the great vessels in the field. Consistent individual variations occur. Preliminary calibrations have given values for stroke volumes and cardiac indices compatible with accepted 'normal' values, but conclusions as to validity await further determinations and simultaneous comparisons with other methods. In addition to determinations of output, study of pattern of the c.d.g. in relation to cardiac disease suggests itself as a means of gaining information regarding mechanical activity of the heart comparable to knowledge of electrical activity obtainable from electrocardiograms.

*Influence of the extrinsic nerves on intestinal motility.*

R. M. WHITROCK (by invitation), H. L. TIECHE (by invitation), AND M. H. SEEVERS. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.

The influence of those reflexes which involve the extrinsic nerves controlling the motility of the small intestine of the dog was studied. The local stimulus of balloon distention was used at various intestinal levels. Dogs were prepared in several different ways utilizing innervated and 'denervated' Thiry-Vella fistulae alone and in conjunction with jejunostomies and ileostomies. Spinal anesthesia was used to block functionally the sympathetic system at the cord. Recordings were made by the balloon-manometer method. The following observations were made: 1) distention of the high jejunum, low jejunum and ileum produces inhibition at all small intestinal levels. 2) The inhibition produced is a reflex effect of extrinsic sympathetic nerves. 3) The sympathetic effect is

purely inhibitory. The parasympathetic effect is purely stimulatory, markedly decreased after vagotomy. 4) Sympathetic sensory afferents from the intestine to the cord do not synapse in the celiac ganglion but pass directly to their respective segmental levels in the cord. 5) Vagal afferent fibers do not appear to carry pain sensation; but rather, pain sensation appears to reach higher sensory levels via the sympathetic system and the spinal cord.

*Asystolic arterial pressure gradient as a measure of changes of local peripheral resistance.* ARNOLD H. WILLIAMS AND HENRY A. SCHROEDER. Dept. of Internal Medicine and Oscar Johnson Institute, Washington Univ. School of Medicine, and Barnes Hospital, St. Louis, Mo.

The total resistance to outflow from the arterial system is composed of a number of resistances in various portions of the body. It is of interest to know the territorial partition of resistance induced by vasoactive drugs and in altered circulatory states such as hypertension. The asystolic arterial pressure gradient is the curve of intra-arterial pressure fall following sudden occlusion of an artery supplying a local circulation. To prove that the gradient is a measure of resistance simultaneous measurements of the gradient and blood flow were made in the femoral or brachial artery of dogs anesthetized with nembutal. Intra-arterial pressure was measured with the Hamilton manometer and flow by a rotameter while collateral blood flow was excluded by wire tourniquets. These gradients did not differ from those obtained under simpler conditions. The combined measurements of pressure and flow furnished an index of resistance with which the changes of the gradient could be correlated. A rough method of estimating resistance changes from the ratio  $\frac{\text{effective pressure}}{\text{rate of flow}}$  was devised. The asystolic

arterial pressure gradient parallels this index of resistance. Therefore the gradient can be used as a measure of resistance. The best method for quantitation that we have found is to measure the slope of the linear portion of the gradient, according to a modification of the Gomez formula for diastolic slope.

*Effects of ryanodine on frog muscle.* J. H. WILLS AND E. F. MURTHA. Pharmacology Section, Medical Division, Army Chemical Center, Md.

Ryanodine lowers the twitch height and alpha excitability of isolated frog sartorii. Ringer's solutions containing from 0.50 to 3.0 mg.% of alkaloid produced also a temporary decrease in the rest length. The effects on twitch height and rest length precede those on alpha excitability, suggesting that the excitable

system in frog muscle is not the same as the one or ones concerned with mechanical responses. The temporary decrease in rest length appears to involve the expenditure of energy, coinciding roughly with a period of increased oxygen consumption.

*Influence of various levels of thiamine intake on maximum work output.* MARJORIE WILSON (by invitation), W. W. TUTTLE AND KATE DAUM (by invitation). Depts. of Physiology and Nutrition, State Univ. of Iowa, Iowa City.

A group of 12 women ate a basic diet containing all nutritional requirements except thiamine. The basic diet contained not more than 140 mcg. thiamine/day. During a 6-week control period all subjects ate an adequate weighed diet as established by the Department of Nutrition. During a 6-week experimental period which immediately followed, 6 subjects ate the basic diet (140 mcg. thiamine) and 6 subjects ate the supplemented basic diet (1340 mcg. thiamine). During both periods measurements of maximum work output, a maximum effort one-minute ride on the bicycle ergometer, were taken. A comparison of the means shows no detrimental effects with respect to work output at the end of 45 days for those eating the low thiamine diet. Because the low thiamine diet produced no change, a similar study was designed to extend over a 19-week period to determine if the element of time was important in the manifestation of thiamine deficiency when work output is used as an index. Three levels of daily thiamine intake were established (200, 625, 1000 mcg.) by supplementing a basic diet containing not more than 200 mcg. thiamine. Three subjects were placed at each level. The subjects whose diet contained 200 mcg. thiamine per day suffered a significant decrease in maximum work output at the end of 19 weeks. The data indicate that there were no significant changes in maximum work output for subjects at the 625 or 1000 mcg. levels of thiamine intake.

*Normal oxygen saturation of human arterial blood during inhalation of air and oxygen.* EARL H. WOOD (with the technical assistance of LUCILLE CRONIN). Section on Physiology, Mayo Foundation, Rochester, Minn.

Roughton and his co-workers (1944) reported that there was a systematic error of approximately 2% in the determination of blood oxygen capacity by the tonometer method, and that this error could be avoided by use of a modification of the method described by Sendroy for determination of blood oxygen capacity directly in the Van Slyke apparatus. Because of the error in the tonometer method, the average value of

95% for normal oxygen saturation of arterial blood, determined by the standard Van Slyke gasometric technic, was 2 to 3% too low, and the resulting value of arterial  $pO_2$  calculated on this basis was approximately 80 mm. Hg, instead of approaching the value of 100 mm. which would be expected if the alveolar to arterial oxygen pressure gradient were very small. These findings have been confirmed in several different laboratories by the use of different methods, and in this laboratory by carrying out simultaneous analyses on a series of blood samples by both the tonometer and Roughton technic. The average normal oxygen saturation of human arterial blood determined in 29 subjects by the technic of Roughton and his co-workers was  $97.9 \pm 0.3\%$ . Arterial saturation was measured in 16 of these subjects by an *in vivo* equilibration technic described by Comroe and Walker. An average value of  $98.6 \pm 0.4\%$  was obtained. Oxygen saturation of arterial blood was determined in 20 subjects during inhalation of oxygen. The average value of  $99.1 \pm 0.2\%$  which was obtained indicates that there was a systematic error in the calculation of oxygen saturation of arterial blood under these circumstances. It is believed that this error, as well as the difference in results obtained by the *in vitro* and *in vivo* equilibration technics, arise at least in part from the calculated correction for physically dissolved oxygen, assuming an insignificant alveolar-arterial oxygen tension difference. The data indicate that the average amount of physically dissolved oxygen during inhalation of oxygen was  $1.83 \pm 0.03$  volumes %, as compared to 1.95 volumes % obtained by the conventional method of calculation. This difference is consistent with an alveolar-arterial oxygen tension difference during inhalation of oxygen of  $40 \pm 9$  mm. and could be explained by assuming that approximately 2% of the blood flow by-passed aerated alveoli. Extensive gasometric calibration studies on the Millikan oximeter, a direct reading oximeter, and a whole-blood oximeter indicate that the relationship between the log of the galvanometer deflection and the oxygen saturation is nonlinear. Therefore, the determination of the increase in arterial saturation during inhalation of oxygen indicated by the oximeter on the standard oximeter scale cannot be used as an accurate indication of normal oxygen saturation of arterial blood.

*Redistribution of electrolytes ( $K^{42}$ ,  $Na^{24}$ ,  $P^{32}$ ) following electroshock convulsions in rats.* DIXON M. WOODBURY. Depts. of Pharmacology and Physiology, Univ. of Utah College of Medicine, Salt Lake City. The rate of uptake and equilibrium concentrations of sodium, potassium and phosphorus by brain and other tissues were studied in control rats and at various

intervals after production of maximal electroshock seizures. The half-times of uptake for brain were: Na 1.2 hours; K two half-times of approximately 2 and 28 hours; and P two half-times of 0.5 and 70 hours. The brain sodium space (or presumptive extracellular space) at equilibrium was determined to be 30% of brain water. The calculated intracellular/extracellular ratios for brain were 28.2 for potassium and 1.77 for phosphorus at 17 hours, but equilibrium was not complete in this period. Immediately following a convulsion there was a reduction of 8.0% in sodium space, which was largely corrected in 20 minutes. At the same time the intracellular/extracellular potassium ratio was reduced by 61%, largely corrected in 20 minutes. The potassium loss from brain cells occurred in spite of a 128% increase in extracellular potassium concentration. A more prolonged increase was found in total brain phosphate, but was associated with a decreased intracellular/extracellular ratio. Although similar changes in sodium space and phosphorus content were found in muscle, liver and spleen, the dramatic loss of potassium was specific for brain. The results suggest an increased permeability of brain cells to potassium during seizures. Urine and plasma determinations of radioactive sodium give evidence that the hypothalamic-posterior pituitary system is activated to cause retention of water and increased sodium excretion for several hours after a seizure.

*Effect of diphenylhydantoin on recovery of various central nervous functions following maximal electroshock seizures in cats.* J. WALTER WOODBURY, JESSE SIMONS, ROBERT EVANS, TRUMAN Y. BURTON AND JAMES E. P. TOMAN. Depts. of Pharmacology and Physiology, Univ. of Utah School of Medicine, Salt Lake City.

Duration of post-seizure depression of various central nervous functions mediated at several levels of integration was measured in ten cats subjected to supra-maximal electroshock stimulation. The recovery times in chronological order are tabulated below for 31 control tonic-clonic seizures in comparison with 33 purely clonic seizures obtained after treatment with diphenylhydantoin 30 mg/kg. i.p. Diphenylhydantoin treatment reduced the duration of depression of all functions studied. The effect was greater for high levels of integration. There was no significant change in order of recovery as a result of treatment. If duration of depression is an index of intensity of previous convulsive activity, then the data suggest that diphenylhydantoin acts at all levels of the central nervous system to reduce the degree of discharge during maximal seizures. The results do not support the contention that the tonic phase of seizures arises at

subcortical levels or that diphenylhydantoin has a predominantly subcortical action.

FUNCTION	TIME TO RECOVERY, SEC		TREATED, %		PROBABILITY
	CONTROL	TREATED	CONTROL	OF	
Knee jerk.....	10.7	6.3	59		0.06
Pinna.....	13.3	12.9	97		0.2
Corneal.....	13.3	8.5	64		0.001—
Normal resp...	13.5	8.2	61		0.01
Pupillary					
light.....	18.3	16.7	91		0.3
Placing.....	35.5	26.7	75		0.001—
Righting.....	47.2	26.5	56		0.001—
Pain with-					
drawal.....	53.3	27.8	52		0.001—
Visual recog-					
nition.....	61.7	40.7	66		0.001—
Rage response					
to pain.....	90.7	68.1	75		0.001—
Normal loco-					
motion.....	108.0	73.3	68		0.001—

#### *Heating effects of microwaves with and without ischemia.*

RALPH E. WORDEN, J. F. HERRICK, KHALIL G. WAKIM AND FRANK H. KRUSEN. Section on Physical Medicine, and Divisions of Experimental Medicine and Clinical Medicine, Mayo Foundation, Rochester, Minn.

Before and after exposure to microwaves, a comparative study was made of the temperatures of the skin, subcutaneous tissue, superficial muscle, and deep muscle of the thigh of the dog with the circulation intact and after artificial ischemia produced by clamping the abdominal aorta. Temperatures produced by periods of 5-, 10-, 15- and 20-minute exposures were measured by means of thermistor and thermocouples. The temperature rises in the ischemic tissues were slightly higher than in the normal tissues but were not considered significant after 5 or 10 minutes of exposure, nor was there any evidence of burning in the experiments performed at these shorter periods. After 15 to 20 minutes of exposure, the increased temperatures in ischemic tissues were considered significant and out of 11 experiments made after exposure for these longer periods, gross evidence of burning was noted in ten. There were several cases in which burning occurred at temperatures that were lower than was seen after exposure of tissues with intact circulation. Temperatures tolerated by normal tissues cannot be regarded as the safe range of tolerance for ischemic tissues. Bony prominences were potential sites for formation of bleb. If an area containing blebs was allowed to cool to control level and again irradiated with microwaves, the temperature of the fluid in the blebs would rise to levels significantly higher than the surrounding

tissues. In addition to the preceding experiments, another aspect of this study was made with trained dogs in order to determine the optimal duration of exposure to microwaves. Periods of 5, 10, 15, 20 and 30 minutes were used. Of these various durations of exposure, the 20-minute period gave maximal heating.

#### *Slow potential changes in the illuminated frog eye. V.*

J. WULFF (introduced by F. R. STEGGERDA). Univ. of Illinois, Urbana.

It has been often postulated that the electrical changes, which occur in a photoreceptor upon illumination, are instrumental in activating the nervous structures in the optic pathway. Investigation of the validity of this hypothesis has resulted in the following observations: 1) In the grasshopper, the retinal electric response begins a short time after the onset of the light stimulus. The discharge of the optic ganglion begins a short time after the onset of the retinal response. Both of these periods of delay vary inversely with the intensity of the stimulating light, when the exposure is constant. 2) In eye-optic nerve preparations (multi-fiber) of *Limulus*, the horseshoe crab, similar observations were made. The results differ in one respect namely, that the inverse relation of the retinal-nerve interval (difference between onset of retinal and optic nerve discharge) does not hold for extremely low intensities of illumination. The magnitude of this interval reaches a maximum at moderate intensities and decreases with higher as well as lower intensities of illumination. 3) In the eyes of the frog it was observed that elevated potentials could be recorded during prolonged periods of illumination. The magnitude of these prolonged potentials increased with increasing intensity of illumination. These observations indicate that, in the frog eye, the initial transient changes are followed by a slow and persistent potential, perhaps analogous to Granit's PI.

These observations satisfy the following predictions of the hypothesis stated above: 1) that the latency of the optic ganglion or optic nerve discharge should increase with decreasing magnitude of the retinal electric response; 2) that the potential generated by the retina in response to illumination persists as long as the optic pathway conducts impulses. The latter prediction is apparently fulfilled in the frog eye. In *Limulus*, however, short but intense flashes of light produces a train of nerve impulse which considerably outlasts the retinal potential.

*The physiology of the adenosine triphosphatase of snake venom.* E. ALBERT ZELLER. Dept. of Pathology, Univ. of Basel, Basel, Switzerland.

Many local reactions, caused by snake bite, can be

connected with well-defined enzymes of the poisons (E. A. Zeller, *Advances in Enzymology* 8:459-491, 1948), but no clear-cut relationships have been established between the paralytic action of many venoms and their enzymes. Investigation of paralytic principles has revealed a powerful ATP-ase in the venoms of all 16 species of snakes hitherto investigated (as well as in scorpion and wasp venoms). The activity varies from species to species, and reaches the highest values in the case of Bitis venoms ( $Q_p = 4000$ ), which are higher than in any other natural source. The heat-labile ATP-ase liberates one molecule of phosphoric acid from ATP. Glycine, veronal or borate solutions form suitable buffers ( $pH = 8.3$ ). Magnesium, cobalt,

manganese, and calcium activate the reaction, while zinc, cadmium, mercury, copper and iron inhibit it. The enzyme is different from similar ATP-ases of vertebrate tissues. It has been shown by Dr. Leya. (Basel) that anaphylactic shock is followed by complete disappearance of the Kurloff-bodies from the blood of guinea pigs. The same happens after the administration of Bitis venom. The addition of zinc (inhibitor) hinders this reaction, as I have shown in experiments performed with Dr. Leya. Thus the ATP-ase of snake venoms seems to be related to the symptoms of shock produced by snake bite. The ATP-ase can be completely inhibited by homologous and heterologous antsnake-venom serums.



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